The Maintenance of Inversion Polymorphism in *Drosophila melanogaster*

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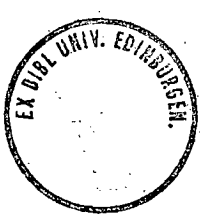

The work which is presented investigated the maintenance of inversion polymorphism in both real and simulated populations. Observations were made of a naturally occurring polymorphism for the common cosmopolitan inversion *In(3R)P* in both inbred and outbred populations of the fruit fly *Drosophila melanogaster*. The results showed a non-significant increase in heterosis, particularly between larval and adult stages, in inbred populations relative to outbred ones. In addition, non-random mating was detected in all populations but there was significantly more disassortative mating in the outbred populations. It is suggested that such phenomena may explain observations documenting the loss or maintenance of inversion polymorphism in experimental populations initiated with different numbers of isofemale lines.

Computer simulation of the maintenance of inversion polymorphism was also undertaken. The modelling examined the development of associative overdominance due to deleterious recessive mutations. The results indicate that such a phenomenon alone is incapable of the maintenance of inversion polymorphism at realistic rates of recombination, mutation and strengths of selection.

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As flies to wanton boys are we to the gods,
They kill us for their sport.

King Lear IV, i, 36-7

Gratefully dedicated to flies everywhere

Contents

Chapter 1: A review of the literature relating to the maintenance of inversion polymorphism in *Drosophila*

This chapter outlines the theory and data on paracentric inversions in *Drosophila*, as a prelude to the studies presented in later chapters. The nature of inversions, their ubiquity in certain taxa and their effects on carriers are briefly considered, as well as the forces maintaining them as polymorphic traits. The literature relating to the use of inversions to reconsruct evolutionary histories and infer phylogenies is not treated.

Most chromosomal structural aberrations fall into one of four main categories: deficiencies, duplications, translocations and inversions (King & Stansfield, 1990). Deficiencies cause a reduction in the amount of genetic material, whereas duplications result in an increase. Neither category has often been found as a polymorphism in natural populations. In the parlance of population genetics a population is said to be polymorphic at a locus if the most common allele at that locus is less frequent than 99 per cent (Christiansen & Feldman, 1986 Ch. 1). This has meant that the study of deficiencies and duplications, at least by population geneticists, has been limited. However *Drosophila ananassae* has been reported as being polymorphic for minute deficiencies and duplications at the end of the chromosomes (Dobzhansky & Dreyfus, 1943). A few (reciprocal) translocations have been found as polymorphisms in natural *Drosophila* populations, but this seems very rare. Translocations cause a change in position of a chromosomal segment within the genome, while the total amount of genetic material remains constant. Inversions are segments of chromosome which have been reversed with respect to the rest of the chromosome. In *Drosophila* this inverted region may form a loop in individuals heterozygous for large inversions, where the inverted and normal homologs pair. However an inversion loop may not appear in other taxa. For example in grasshoppers there is straight pairing of homologous chromosomes (White, 1977). If we consider all animal taxa inversion polymorphism is a rare phenomenon. This

is because polymorphism can only arise in species that have mechanisms to avoid the consequences of crossing over in heterozygotes. Then some form of balancing selection, involving heterosis or frequency-dependent selection, can lead to a balanced polymorphism. In species lacking these mechanisms inversions will usually be lost from a population but can become fixed if one invokes associated segregation distortion or random genetic drift in small populations. These aspects of chromosome evolution will be dealt with later in the appropriate sections.

There are two types of inversion: pericentric inversions which include the centromere, and paracentric which exclude it. Pericentric inversions are rare in natural populations, largely because of the consequences of single exchange crossovers within the inversion loop in inversion heterozygotes (heterokaryotypes). This kind of recombination generates monocentric chromosomes which are duplicated or deficient. Since there appears to be no mechanism to exclude them from functional egg nuclei, they result in aneuploid zygotes that normally die (Roberts, 1976). Some of the earliest work on inversions in *D. melanogaster* proposed the resulting decrease in fertility for female pericentric heterozygotes as the explanation for the rarity of naturally occurring pericentric inversions (Sturtevant & Beadle, 1936). In support of this view, whenever pericentric inversions have been found a mechanism has also been present to reduce the probability of exchange within it, such as the localisation of exchange or an overlapping paracentric inversion (Sperlich & Pfriem, 1986; Coyne *et al*, 1991).

Paracentric inversions produce abnormal chromatids through single crossovers within the inversion loop. However, the consequent dominant lethality seen with pericentric inversions is avoided because these chromatids are selectively eliminated from the functional products of female *Drosophila* meiosis, and are isolated in the polar body nuclei. Other species of Diptera have analogous mechanisms to avoid the heterozygote disadvantage caused by production of aneuploid gametes. The functional egg pronucleus will therefore receive only normal, nonrecombinant chromatids.

This means that in effect the inversion supresses recombination, as there is normally none in *Drosophila* males (Ashburner, 1989). Multiple crossovers can occur, however, and their products are recoverable but relative to single crossovers they are rare due to positive interference (Roberts, 1976).

The suppression of recombination achieved by an inversion in *D. melanogaster* is dependent upon its length (the longer it is the greater the suppression), its position on the chromosome (the more distal the greater the suppression) and whether other chromosome arms are heterozygous for inversions (Krimbas & Powell, 1992). Some studies have found nonnegligible rates for double crossovers, such as for the loci *h* and *e* which are near the middle of the cosmopolitan inversions *In(3L)P* and *In(3R)P*. In heterokaryotypes these loci recombined at a rate of about 2×10^{-4} (Payne, 1924). Gene conversion and mutation may also generate variation in an originally monomorphic inversion. The former mechanism has been studied for the *rosy* locus within an inversion covering about 65% of the 3R chromosome arm in *D. melanogaster* ($In(3R)P_{18}$). The rate of exchange of this gene in heterokaryotypes was 1.34×10^{-5} (Chovnick, 1973). The most recent estimates for mutation rates at *D. melanogaster* allozyme loci within inversions are between 1×10^{-6} and 4×10^{-6} (Voelker *et al*, 1980). When heterozygous, paracentric inversions also increase the frequency of recombination between nonhomologous chromosome pairs and of chromosome non-disjunction. The frequency of recombination in individuals homozygous for a paracentric inversion is about the same as in the wild type (Ashburner, 1989).

Inversion polymorphism exists in more than two thirds of the *Drosophila* species that have been studied (Sperlich & Pfriem, 1986). In fact it has been recorded across most families of the order Diptera, and in other insects such as grasshoppers and species of Collembola (White, 1977 Ch. 8). A much lower percentage of mammalian species show chromosomal polymorphism generally, but examples of inversion polymorphism have been found in populations of *Rattus rattus*, *R.*

norvegicus (Yosida *et al*, 1965) and *Peromyscus maniculatus*, the North American deer mouse (Ohno *et al*, 1966).

Although the polytene chromosomes found in certain tissues in Diptera had been studied since 1881 (see White, 1977 Chap 4 for brief history), they could only be examined in detail after the development of the salivary gland technique in *Drosophila* by Painter (1933). Soon afterwards chromosomal inversions were first observed (Tan, 1935) and by the mid-1940s their frequency in natural populations had been measured (eg. Dobzhansky, 1944). Over the next three decades Dobzhansky and his collaborators also described the forces governing the dynamics of inversion polymorphisms, particularly in *D. pseudoobscura*. His work will be discussed in later sections. As a result of this early start, paracentric inversion polymorphisms in *Drosophila* are some of the most extensively studied polymorphisms both in nature and in the laboratory. The purpose of this review is to examine the various forces which may play a part in the fine balance that maintains such a polymorphism. These forces are summarised in Table 1.

Table 1

<table>
<tr><td rowspan="4">Deterministic advantage</td><td rowspan="3">Selection</td><td rowspan="3">Balancing selection</td><td>Heterosis</td></tr>
<tr><td>Frequency dependence</td></tr>
<tr><td>Environmental heterogeneity</td></tr>
<tr><td>Recombination modification</td><td></td></tr>
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<tr><td>Drift</td><td></td><td></td><td></td></tr>
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The table makes an initial division between theories relying on random genetic drift and those dependent on any kind of deterministic advantage the inversion may confer upon carriers. The latter category is further divided to define theories involving meiotic drive and natural selection on the inversion. Hereafter the categorisation becomes somewhat more arbitrary. It could for example be argued that because recombination modification occurs in heterozygotes it is a special case of overdominance. I have given it a separate category because the advantage it confers (of reducing crossing over between selectively advantageous genes) manifests itself at the population level. There is also some overlap between frequency-dependent selection and environmental heterogeneity. This is especially true of spatial heterogeneity and will be more fully discussed later.

Heterosis

The term heterosis (or hybrid vigour) was originally used to describe the improved growth, survival and fertility of crosses made between inbred lines. Yields of corn produced by such crosses are typically 15% to 35% above those of outcrossing varieties (Hartl & Clark, 1989 Ch. 5). Heterosis is always associated with increased heterozygosity (King & Stansfield, 1990). Probably for this reason the terms heterosis and heterozygote advantage (see below) are sometimes used synonymously (eg. Maynard Smith, 1989). This is the approach which will be followed here.

Heterozygote advantage is the situation where heterozygotes at a locus are inherently fitter than either homozygote. This situation can lead to a balanced polymorphism. For example at a locus with two alleles (A, a) polymorphism may be maintained because either allele will increase in frequency when rare (Hartl & Clark, 1989 Ch. 4). When the A allele is rare the population will consist mainly of aa homozygotes and some heterozygotes. In other words the A allele will now be present in individuals of above

average fitness. This will lead to an increase in the allele's frequency, and of course the process would be the same when allele *a* is rare.

There is little direct evidence for the presence of heterotic loci in natural populations. However very low, effectively undetectable differences (of 1 per cent or less) in the fitnesses of genotypes would be sufficient to maintain polymorphism (Maynard Smith, 1989 Ch. 4). Heterosis was originally demonstrated in *Drosophila* by Gowen (1952) amongst others, who measured viability and fecundity in lines which had undergone various levels of inbreeding. Using balancer chromosomes Dobzhansky & Spassky (1954) showed that chromosomal heterozygotes survive and reproduce at significantly higher rates than homozygotes, although the selective differentials were not large. This approach was further developed to produce the 'balancer equilibration' (BE) technique (Sved & Ayala, 1970) which gives an overall estimate of chromosome homozygote fitness. This technique has been used in studies of several *Drosophila* species and chromosomes; in every case the results have indicated that homozygotes suffer a substantial depression in fitness relative to heterozygotes (Simmons and Crow, 1977).

Perhaps the simplest mechanism involving heterosis for the maintenance of inversion polymorphism is the 'position effect hypothesis'. This theory asserts that after the appearance of a new inversion heterotic position effects are the only factors responsible for the establishment of a new polymorphism. When the inversion has risen appreciably in frequency, natural selection may act to favour gene combinations that improve the existing heterosis (Mainx *et al*, 1953). There is some evidence for this idea from laboratory studies of X-ray-induced inversions in *D. pseudoobscura* (Sperlich, 1966). However the majority of the evidence from studies of the fate of new inversions emphasises the importance of the gene content of the inversion, and its ability to be heterotic with other gene arrangements in the population (reviewed by Krimbas & Powell, 1992).

Many studies of *Drosophila* in nature and the laboratory have shown heterosis associated with inversions: this is true of studies of male mating sucess in *D. subobscura* for certain inversions (Krimbas, 1992). Female *D. melanogaster In(2L)t* heterokaryotypes have been shown to carry significantly more sperm than homokaryotypes, and to have an advantage in female productivity (defined as the mean number of offspring from a female) (Watanabe & Watanabe, 1973). In the same species a heterokaryotype advantage has been demonstrated in egg to adult viability for the third chromosome inversion *In(3R)P* in the presence of the pesticide DDT (Barnes & Merrel, 1985). The dynamics of the well documented *D. pseudoobscura* third chromosome polymorphism seem equally or better explained by frequency dependence, as opposed to heterosis (Wright, 1977 Ch. 9).

When many enzyme loci are examined it is possible to find a positive correlation between the number of heterozygous loci in an individual and an important fitness trait such as growth rate (Mitton & Grant, 1984). These observations can be attributed to either heterotic selection at the protein loci themselves (genuine overdominance) or to selection at closely linked loci (associative overdominance). Nei (1987 Chap 8) has convincingly argued that the available data for heterotic protein loci is best explained by associative overdominance. He envisaged linkage disequilibrium built up by genetic drift in a finite population resulting in a kind of associative overdominance. This takes the form of many deleterious genes closely linked to a protein locus with two selectively neutral alleles, heterozygotes at this locus will tend to show a higher fitness than homozygotes (Ohta, 1971). The major problem of this model is that the associative overdominance may disappear if recombination takes place between the protein locus and the neighbouring deleterious genes. However this problem is avoided if all the loci involved are associated with an inversion so that no recombination takes place between them. Wilton *et al* (1989) performed a set of experiments designed to test a prediction of the associative overdominance hypothesis in *D.*

melanogaster. The prediction was that if this hypothesis reflects the real situation then it should be possible to select out the deleterious genes, and derive 'purged' homozygote lines having fitness equal to the heterozygote. Population cages containing just two wild-type chromosomes were set up and ran for many generations, single chromosomes were then isolated from the cage and had their fitnesses measured by the BE technique. They were unable to detect any unequivocal increase in fitness, but the results did not allow them to completely reject the associative overdominance model. Associative overdominance could have been operating but the results constrain the parameters of such a model to a narrow range.

Two of the effects generally thought to produce the heterosis associated with *Drosophila* inversion polymorphisms are effectively the same as genuine and associative overdominance (Maynard Smith, 1989 Ch. 4). The first, 'genic heterosis', is the case where inversion heterozygotes are also heterozygous for heterotic alleles. 'Chromosomal heterosis' on the other hand is a result of both the inverted and non-inverted sequences carrying deleterious recessive mutations. This is the reason that the heterotic inversion polymorphisms of the Diptera need not necessarily be equated with the presence of heterotic genes.

The heterosis seen with inversion polymorphisms may also involve a third effect: 'coadaptation'. This is the theory that the selective value of an inversion depends on the combination of alleles it protects from separation by recombination, in the following way. Epistatic fitness interactions act such that a gene arrangement has particularly high fitness in heterozygous combination with other arrangements in the same population. The evidence for and against this kind of epistatic heterosis is reviewed by Powell (1992). However as the theory relates to the adaptation of inversions to disparate ecological niches, it will be dealt with later.

Frequency-dependent selection

When rarity of phenotype is a selective advantage, the direction of selection depends on the gene frequency. That is, a gene producing the phenotype is selected for at low frequency and against at high frequency, resulting in a balanced polymorphism (Falconer, 1989 Ch. 2). As early as 1930 Fisher had made this point in relation to mimicry in butterflies (for the historical development of the concept since then see the review by Ayala & Campbell, 1974). Predation would be expected to produce such an effect when a predator forms a 'search image' of a common type of prey. This is because rare individuals, not conforming to this image, are more likely to escape predation (Futuyma, 1986 Ch. 6).

Host-parasite coevolution has traditionally been seen as an 'arms race' in which each protagonist experiments with novel methods of defence or attack. With reference to a host, a new method of defence may 'cost' more than its existing method, for example by reducing the available resources for reproduction. This will mean that the new defence method is of net benefit only if the host is likely to be attacked by a parasite against which there is no 'cheaper' defence. So the relative fitness of a defence depends on the frequencies of the various parasitic attacks. The parasites face an analogous problem in finding new methods of attack (Hedrick, 1983 Ch. 13). The second, simpler case of host-parasite generated frequency-dependence is when there are no differences in cost between particular methods of defence or attack. It is merely that certain defences best counter certain kinds of attack and *vice versa*. Here each species constitutes a variable environment for the other, and again the relative success of a strategy is determined by the frequencies of the strategies utilised by (or the genotypes of) the other species (Seger & Hamilton, 1988). In either of the two ways just outlined parasites may cause the accumulation of polymorphic variation in host populations. Whether parasites in nature actually do is largely an unanswered question. The existing evidence

(reviewed by Seger & Hamilton, 1988) is certainly inconclusive, but it does seem favourable to the idea that host population polymorphism can be maintained by parasites.

Competitors of the same species may also be involved in the exertion of frequency-dependent selection. High numbers of individuals of one morph can result in more competition or noxious by-products, thus favouring another type with different resource requirements. In this way individuals with differing ecological needs can coexist together (Hartl & Clark, 1989 Ch. 9). This density-dependence has been sought many times for *Drosophila* enzyme loci in the context of larval viability. As a result of this work evidence exists both for (e.g. Dawood & Strickberger, 1969) and against (eg. Yamazaki, 1971) the phenomenon. However some doubt has been expressed that larval resource requirements could be sufficiently finely tuned to maintain a significant degree of the substantial polymorphism observed (Maynard Smith, 1989 Ch. 4).

In addition, female choice may be expected to produce a frequency-dependent sexual advantage for a preferred male phenotype (O'Donald & Majerus, 1988). Negatively frequency-dependent male mating success (the "rare-male effect") has been documented in a number of *Drosophila* laboratory experiments. For example, in *D. pseudoobscura* the rarest genotype reportedly gains a mating advantage in competition in mixed populations, where the rare genotype is characterised by visible gene markers, polygenic traits or different gene arrangements (Ehrman & Spiess, 1969). There is also some evidence in the same species that frequency-dependent mating success (and viability) may be involved in maintaining the third chromosome gene arrangement polymorphism in natural populations (Anderson, 1989). In *D. melanogaster* the third chromosome inversion *In(3R)P* also shows balancing frequency-dependent selection, at least under laboratory conditions (Nasser *et al*, 1973). There is no convincing evidence for frequency-dependent female preferences as a mechanism for the rare-male effect, and

male competition seems only to be important in certain cases. As a result it is now thought that the most likely explanation for these effects is a mixture of fixed female preferences (Partridge, 1988). Evidence consistent with this exists for the seaweed fly (*Coelopa frigida*) inversion polymorphism, which suggests that females may choose mates on the basis of inversion karyotype (Engelhard *et al*, 1989); and for *D. melanogaster* wild-type females with respect to increased mating with mutant males (Heisler, 1984).

Environmental Heterogeneity

An environment which varies spatially or temporally can, in the absence of heterozygote advantage, account for the maintenance of a polymorphism. Haldane (1930) was the first to derive a theory for the maintenance of polymorphism as a result of spatial variation. The idea is basically that different alleles at a locus are favoured in different adjacent environments, with limited migration occurring between the populations occupying these environments. For a wide range of fitness differences between alleles and migration rates, each local population receives an input of genes which are favoured (and therefore at high frequency) elsewhere. This process can result in a stable genetic polymorphism in each population (Falconer, 1989).

The considerable modern literature on polymorphism and spatial variation (reviewed by Seger & Brockmann, 1987) started with Levene's (1953) classic paper. In Levene's model density-dependent regulation of population size occurs in the local populations, and there is free migration such that each local population contributes a fixed number of adults to the global mating pool. The number of adults contributed by a local population is irrespective of the average fitness of the population of origin, and for this reason 'soft selection' has been described as operating. A form of allelic frequency dependence then appears at the level of the global population (Walsh, 1982). Maynard Smith and Hoekstra (1980) showed that the models of Levene

(1953) and Dempster (1955: see below) rely on large selective advantages per locus or on the relative niche sizes lying in a narrow range. This cast serious doubt on the plausibility of these mechanisms as important causes of polymorphism. Levene's model can be modified in several ways to make it more successful in maintaining polymorphism (eg. Gillespie, 1981; Seger, 1985). In the light of these modifications it would seem that polymorphism may arise due to spatial variation, as simple patterns of selection, migration and population regulation give rise to the frequency dependence already mentioned. That is to say, each allele is favoured in one environmental niche but may be unhelpful or even lethal to an organism in another niche. The marginal fitnesses at gene frequency equilibrium are however the same (Seger & Brockmann, 1987).

There is good evidence for the maintenance of inversion polymorphism involving spatial variation in *D. pseudoobscura*. The clines in gene arrangement frequency that Dobzhansky (1970) and his coworkers found were shown to be attributable to natural selection favouring one arrangement in one locality, and another in a different locality. Latitudinal clines in the seven cosmopolitan inversions of *D. melanogaster* found across various continents would also seem to be attributable to spatial environmental variation (Sperlich & Pfriem, 1986).

Models have also been devised which explore the potential of temporally fluctuating environments to maintain polymorphism, where selection coefficients vary with time. This work started with Dempster's (1955) haploid model with discrete generations. He showed that the relevant measure of fitness is the geometric mean (the nth root of the product of a set of n positive numbers) in a temporally varying environment. (This is the correct measure because long-term fitness, like population growth, is inherently multiplicative rather than additive.) Dempster found that, unlike the simplest models of spatial variation, his model for temporal variation could not maintain polymorphism. The exception to this is when overlapping generations and iteroparity

are introduced, which is analogous to allowing migration between niches or generations. The result is a more 'Levene-like' model which is capable of maintaining polymorphism. The diploid case was studied by Haldane & Jayakar (1963) who found that their model could also maintain polymorphism. This latter model eventually led to the discovery of the general condition, under temporal variation, for polymorphism: the geometric mean fitness of the heterozygote must exceed that of either homozygote (Karlin & Liberman, 1974). Theory relating to temporally fluctuating selection has since progressed in various directions. One interesting finding is that the expected distribution of gene frequencies, under certain patterns of fluctuating selection and mutation, may mimic that expected through the action of drift and mutation (Gillespie, 1978). In general it seems that spatial is more effective than temporal variation in maintaining polymorphism, at least for short-lived animals with few reproductive episodes (Seger & Brockmann, 1987). Some models have combined spatial and temporal environmental variation but they have proved to be complex and difficult to analyse. For example Levins (1968) concluded that, depending on which type of variation was most important, the results would be similar to those for spatial or temporal variation alone.

The work of Dobzhansky and his colleagues (e.g. Dobzhansky, 1956) has, as in the case of spatial variation, furnished a well documented example of maintenance of polymorphism involving temporal variation in *D. pseudoobscura*. They found that various local populations underwent cyclic seasonal changes in the relative frequencies of various gene arrangements. These changes were in accordance with the observed effects of spatial variation: certain arrangements were most frequent in summer and at sea level, while others were more common in winter or at high altitudes. It proved possible to reproduce this form of directional selection in the laboratory (reviewed by Dobzhansky, 1961).

Matters were complicated by observations of remarkable changes in gene arrangement frequencies superimposed on the aforementioned cyclic changes. In populations of *D. pseudoobscura* across California, Nevada and Arizona, Dobzhansky (1958) saw surprisingly rapid responses to environmental change in gene arrangement frequencies. He described the forces behind these responses as blends of directional and balancing selection. One example was the arrangement PP, which was recorded in only 4 out of almost 20, 000 third chromosomes between 1940 and 1945. By 1957 the frequency of these chromosomes in the same localities was nearly 8 per cent of the total sampled. Over the same period the frequency of other gene arrangements (especially CH and SC) fell by comparable amounts. After 1957, up to 1963 the ST arrangement rose in frequency, accompanied by a decrease in almost every other arrangement. These changes were so striking that the effects were at first attributed to the widespread use of DDT and other insecticides. However it was discovered that frequency changes were not particularly pronounced in and near agricultural areas. In addition changes in frequency were no less remarkable in uncontaminated areas. Another explanation was suggested: that the changes were adaptive reconstructions of these populations. In this view balancing selection reconstructs the gene pool of a population in response to a new chromosome arrangement (arising by mutation or recombination) which produces highly fit heterokaryotypes with certain other pre-existing arrangements in the same population. The frequencies of these complementary chromosomes increase, while others are correspondingly reduced. This reconstruction in frequencies then sweeps over the area in which the new coadapted system suits the prevailing environment (Dobzhansky, 1963).

Recombination modification

In the controversy surrounding theories on the evolution of sex most effort has been exerted in finding reasons for the presence of recombination, rather than its absence. From these

discussions two main explanations for the maintenance of recombination have evolved. One of these theories suggests that recombination is present as a side-effect of DNA repair mechanisms (Bernstein *et al*, 1988). In the view of these authors the fact that recombination may provide new genetic variability is an unselected consequence of the presence of DNA repair genes. The opposing explanation is that the production of new gene combinations is the most important force in the maintenance of recombination. For example in the opinion of Maynard Smith (1988) the main short-term selective force involved is a result of recombination allowing changes in the value of the linkage disequilibrium between fitness loci. These changes are necessitated by selection for a shifting optimum value of a polygenic trait, which may be expected in a fluctuating environment. Some related theories see this linkage disequilibrium as a result of random genetic drift as opposed to selection (Felsenstein, 1988).

Fisher (1930) first suggested that selection may favour closer linkage between two alleles which are advantageous in combination. The theoretical consideration of inversions simply as recombination modifiers became an area of interest much later. A reasonably complete picture of the fate of an inversion introduced into a two-locus system with low recombination has been built up in theoretical studies following Fisher's early work. Feldman (1972) and Charlesworth and Charlesworth (1973) independently came to the conclusion that there will be selection for a recombination modifier only when it occurs in a gamete whose marginal fitness is higher than the population mean fitness. That is, an increase in the recombination modifier's frequency in the population requires linkage disequilibrium between the loci which the inversion is to include.

Deakin (1972) examined the several equilibrium states it is possible for an inversion to reach, and Charlesworth (1974) looked at the stability of these equilibria. The latter author found that an important factor in the stability of the equilibrium

is the nature of the fitness interactions between the two loci. With certain fitness relationships there is more than one possible equilibrium and the final frequency of the inversion depends on the population history. This conclusion is consistent with the data of Watanabe *et al* (1970) on *D. pseudoobscura*. Later work extended the theory to include the fate of recombination modifiers in clines (Charlesworth & Charlesworth, 1979). It was found that selection for increased recombination can occur but it is generally weaker than selection for decreased recombination. Charlesworth (1976) also looked at selection on recombination modifiers in a fluctuating environment where the sign of the linkage disequilibrium (D) changes. It seems that it is fluctuation in the sign of D which causes selection for increased recombination and, in this latter model against the modifier. This is to be expected as recombination can achieve only one thing: the reduction of D. So if recombination is to be advantageous then there must be epistatic fitness interactions between loci whose values of D periodically change sign, because of drift or changing selection, as mentioned above (Felsenstein, 1988).

The fact that environmental periodicity and variation can increase selection for recombination helps us interpret some comparative data in genetics. This may apply for example to Carson's (1965) data on certain *Drosophila* species. He found lower levels of heterozygosity in ecologically marginal populations compared with central populations and speculated that this was due to selection for increased recombination because of greater environmental fluctuation.

Charlesworth and Charlesworth (1980) went on to show that there can be selection for decreased recombination in the special case of sex chromosomes. This occurs between the sex locus and a second selected locus if there is already a selectively maintained sex difference in allele frequency at the second locus. This proposition is in agreement with Post's (1985) work on sex chromosome differentiation by the accumulation of inversions in the blackfly *Simulium erythrocephalum*.

The above papers, in common with most on the subject of recombination modifiers consider the fate of those modifiers with no direct effects on fitness. As already mentioned, this situation only seems to occur commonly in the case of Dipteran paracentric inversions. The more common case where the inversion not only changes the linkage between loci but also decreases heterozygote fitness has had less attention. Bengtsson and Bodmer (1976) obtained results for an inversion with heterozygote disadvantage which are similar to those derived for selectively neutral inversions. Factors found to determine its fate were the linkage disequilibrium between the loci; the fitness relationships between the loci; and also the amount of recombination between segregating loci in individuals of standard karyotype. These assertions remain untested empirically however.

Meiotic drive

Meiotic drive can broadly be described as the process through which Mendelian segregation becomes biased, usually because of the differential production of gametes by heterozygotes (Crow, 1986 Chap. 7). For example in both *Mus musculus* and *Mus domesticus* a tightly linked group of genes called the t complex exhibits grossly distorted segregation. Male mice heterozygous for the t complex have been shown to transmit it to over 90% of their offspring (Hartl & Clark, 1989 Chap. 4). Another classic example of meiotic drive is the segregation distorter locus (Sd) in *D. melanogaster*, and here more is known about the molecular mechanisms underlying the biased segregation. In this case an allele of the Sd locus acts to destroy gametes bearing a certain allele at the responder (Rsp) locus. It seems from recent work that the Sd product prevents normal chromatin condensation at the Rsp locus, which is now known to be a satellite DNA array (Doshi *et al*, 1991).

Both the t complex and Sd alleles seem to be maintained as polymorphisms by natural selection against their homozygotes

balancing meiotic drive in heterozygotes (Hedrick, 1981). In other words natural selection, at the level of the individual, counters the lower level selection favouring the meiotic drive gene. In Gould's (1983) view selection may operate at various levels of organisation hierarchically, at the level of genes, individuals, groups and species. It is possible to imagine other examples where polymorphism is maintained by a balance between selection at different levels and in opposing directions (Ohta, 1992).

The best example of an inversion polymorphism which partly relies upon meiotic drive for its maintenance is the sex-ratio (SR) gene arrangement. In several *Drosophila* species (most belonging to the *obscura* group) the SR condition is due to the presence of one or more inversions on the X chromosome relative to the standard arrangement (ST). Males carrying SR exhibit biased segregation of the X chromosome, to the point where offspring are mainly or exclusively female. Segregation in females which are heterozygous for SR conforms to the normal Mendelian case (Krimbas & Powell, 1992). It appears that this situation arises as a result of males hemizygous for SR producing only or predominantly X-bearing functional sperm. Sperm receiving a Y chromosome degenerate, or in some species are not formed at all (Policansky & Ellison, 1970). Paradoxically though, SR males have been shown to be as fertile as ST males in the laboratory.

With regard to the selective forces maintaining it, the best studied SR polymorphism is that of *D. pseudoobscura*; although this literature (reviewed by Powell, 1992) is often contradictory. In this species the SR arrangement reaches frequencies of 20% or less in natural populations, so there must be one or more forces opposing its drive to fixation. There is evidence which suggests that there is a heterotic effect of SR on female fertility (Curtsinger & Feldman, 1980). Also it would seem that males carrying SR suffer a mating disadvantage; engaging in fewer multiple inseminations resulting in the displacement of their sperm by ST males (eg. Wu, 1983). Theoretical simulations

suggest that these male virility deficits for SR males are sufficient to maintain the polymorphism (Wu, 1983). There is also somewhat more equivocal evidence for viability differences between karyotypes (see aforementioned Powell, 1992 review).

It has been established that meiotic drive is capable of explaining the fixation of a new underdominant inversion (Bengtsson & Bodmer, 1976; Hedrick, 1981; Walsh, 1982). However there is no evidence for this, in fact the only documented case of meiotic drive associated with a chromosomal rearrangement drove in the wrong direction (White, 1978). The only experimental work done to look for fixed chromosome differences involving meiotic drive gave negative results (Coyne, 1989). In addition there is no evidence for fixed meiotic drive alleles among closely related species, and the parapatric geographic distribution of some chromosome arrangements could not occur if they were meiotically driven (Coyne, 1989).

Random genetic drift

Thomson (1977) has described a theory whereby inversions are seen as "the visible result of hitchhiking". It states that on the rare occasions that an inversion occurs so that it contains one or more selected genes, the frequency of the whole inverted region will increase merely as a result of an increase in the frequency of the selected genes. This view is apparently supported by some circumstantial evidence from studies of linkage disequilibrium in *Drosophila*: most associations found have involved inversions. In Thomson's view the disequilibrium is built up by hitchhiking, that is by random drift (the initial chance association of a selected gene with the inversion) followed by selection. Recombination suppression is seen as only serving to prolong the linkage disequilibrium produced. Thomson sees her theory as mainly relevant to the establishment of new inversions, and admits it is hard to see how this phenomenon could maintain polymorphism without an opposing force.

In common with most major chromosomal rearrangements, inversions substantially reduce the fertility of heterozygotes in most species. Despite this, inversions have become fixed in vertebrate lineages at a rate of roughly 10^{-6} to 10^{-7} per generation, and in invertebrate lineages at a rate an order of magnitude more slowly on average (Lande, 1979). Most work on the spread of inversions as a result of drift has centred on this apparent paradox: the fixation of underdominant inversions.[1]

White (1977, Chap. 8) assumed a substantial heterozygote disadvantage of the same order of magnitude or larger than the expected inbreeding depression (through the accumulation of deleterious recessives within the inversion) of the inversion homozygote. Taking into account the larger variance in fitness of inversion homozygotes than the heterozygotes it is much more likely for homozygosity rather than heterozygosity to be beneficial. This is likely to be the main reason that large inversions are usually found as fixed differences between races and species, rather than as stable polymorphisms.

Wright (1941) first discussed the probability of fixation of a chromosomal rearrangement, namely a reciprocal translocation. He concluded that fixation was most likely to occur by drift in a small, isolated population prone to frequent extinction and recolonisation. This work was extended to include inversions by Bengtsson and Bodmer (1976) and Lande (1979, 1984). Lande (1984) found the fixation rate of chromosomal inversions to be extremely sensitive to three factors: the amount of genetic variance in fitness; the correlation in the fitnesses of heterozygotes and homozygotes; the population structure. He showed that the population structure most conducive to the fixation of negatively heterotic inversions is composed of "small, nearly isolated local populations or demes, with effective sizes on

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[1] It should be noted that work by Coyne (1991) questions the degree to which inversions that become fixed in nature are underdominant. If it is those inversions that are only slightly underdominant that are fixed then mechanisms such as meiotic drive and strong episodes of drift become unneccesary to explain fixation.

the order of 100 individuals or less, and high rates of local extinction and colonisation." In such populations most inversions are expected to be fixed by drift despite heterozygote disadvantage. In contrast the fixation of rare advantageous rearrangements: selectively beneficial to heterozygotes and homozygotes, is favoured in large stable populations. Thus it would seem (in the absence of evidence for fixed advantageous rearrangements) that random drift may play a significant role in the spread of inversions through populations.

The theory that inversions incurring heterozygote disadvantage can become fixed by drift in small populations is supported by the following indirect evidence. The negative correlation in animals between rate of chromosomal evolution and heterozygosity (Coyne, 1984) suggests that small populations have been the rule in the past. In addition it is likely that population bottlenecks have occurred in species which now have large populations (Wright, 1978). Evidence of the importance of inbreeding acting in concert with drift comes from the *Clarkia* plant species (Lewis, 1973) and rodent populations (Bush *et al*, 1977). Both of these organisms undergo relatively high inbreeding in small populations and are known for high rates of chromosomal evolution.

The following chapters investigate some of the phenomena that play a role in the maintenance of a well known inversion polymorphism in *D. melanogaster* populations. In particular, an attempt is made to assess the importance of chromosomal heterosis in these populations and to unravel interactions between fitness components. In addition, computer modelling is undertaken to complement the conclusions drawn from experimental results.

Chapter 2: Heterosis and non-random mating in inbred *D. melanogaster* associated with the cosmopolitan inversion *In(3R)P*

Assuming all new inversions appear as a single copy, some initial advantage to heterokaryotype carriers is necessary for the inversion to become established. This heterosis may involve three separate phenomena. The first two are effectively the same as genuine and associative overdominance. 'Genic heterosis', is the case where the inversion heterozygotes' advantage results from heterotic alleles, as with genuine heterosis. 'Chromosomal heterosis' on the other hand is a result of both the inverted and non-inverted sequences carrying different deleterious recessive mutations (Maynard Smith, 1989 Chap 4). A related concept was suggested by Dobzhansky (1970) to explain his own observations of experimental *D. pseudoobsura* populations. This is a kind of epistasis, acting so that different gene arrangements in the same population become 'coadapted' and behave heterotically in combination with each other. However it is not claimed that such coadaptation could maintain a polymorphism.

There are many documented cases of heterosis associated with inversions in *Drosophila* species. Studies of male mating success in *D. subobscura* have revealed heterotic effects for certain inversions (Krimbas, 1992). Most work on *D. melanogaster* has examined the polymorphism for the second chromosome inversion *In(2L)t*. At certain (rather high) temperatures the karyotypes containing this inversion had higher survival rates as larvae and pupae than the standard homokaryotype (Van Delden & Kamping, 1989). Males with karyotypes containing *In(2L)t* have been shown to mate with more females than males lacking the inversion. Other studies have found significant advantages to heterokaryotypes in female productivity (defined as the mean number of offspring from a female) (Watanabe *et al*, 1976). Much less work has been done on the *In(3R)P* polymorphism, but heterosis in egg to adult viability has been demonstrated in the presence of the pesticide DDT (Barnes

& Merrel, 1985).

In the case of the polymorphism for third chromosome gene arrangements (consisting of a series of inversions) in *D. pseudoobscura*, differential male mating success may be the most important fitness component involved in its maintenance (Anderson & Watanabe, 1974). This certainly seems true of natural populations where male mating success (and especially its subcomponent, male fertility) is the only fitness component documented to be acting. Male mating success includes the ability to court and copulate as well as characteristics like the propensity to mate and remate. Other factors, such as sperm competitive ability are omitted.

Negatively frequency-dependent male mating success (or the rare-male effect) has often been investigated in *Drosophila* because of its potential to maintain polymorphism with no cost in terms of genetic load. However, as Partridge (1988) has pointed out, to achieve this effect the mating advantage must be countered by opposing selection of some kind. This may take the form of the mating advantage changing to favour another male morph at some intermediate frequency, or of negative selection on a different fitness component so as to achieve selective equality between morphs. Unfortunately the rare-male effects that have been described (and that are not likely to be artefacts of the experimental designs employed) are often found in the absence of such an opposing selective force (Partridge, 1988). Nevertheless it has been shown that the dynamics of the *D. pseudoobscura* third chromosome polymorphism are equally or better explained by frequency dependence, as opposed to heterosis (Wright, 1977, Chap. 9). There is some evidence from *D. pseudoobscura* that frequency-dependent viability as well as mating success may be involved in maintaining the polymorphism (Anderson, 1989).

The polymorphism for gene arrangements in *D. pseudoobscura* was first investigated by Dobzhansky and Levene (1948). They demonstrated indirectly that mating with respect to karyotype was random by comparing the karyotypic frequencies in eggs laid by wild females with Hardy-Weinberg (H-W) expectations. It was later shown that the ability of such comparisons to detect departures from Hardy-Weinberg frequencies due to selection is limited (Lewontin & Cockerham, 1959). The supposition that net fitness could be measured by the comparison of genotypic frequencies from some point in the life cycle with the H-W frequencies predicted by the same genotypic frequencies in the previous generation was common at this time. However, in his classic paper Prout (1965) showed that this supposition was false as it did not take account of differential fertility. Lewontin (1974) has illustrated Prout's argument as follows. If one imagines a population in which a balanced lethal system operates at the egg stage such that only adult heterozygotes are found in the first generation, then the estimate of gene frequencies from these adults will be p=q=0.5. The predicted H-W ratio in the second generation will be 0.25AA: 0.5Aa: 0.25aa assuming no selection. The balanced lethality present will result in only heterozygotes in the second generation, and the fitnesses of the homozygotes will be correctly estimated as zero. Alternatively if one imagines a population in which there is an analogous situation of balanced sterility this method becomes untenable. The three genotypes will appear in each generation in the predicted 1: 2: 1 ratio and equal genotypic fitnesses (or no selection) will be estimated, even in the presence of this severe heterosis for fertility.

Direct analysis of mating patterns by examining the frequencies of matings between karyotypes provides a more powerful method for detecting non-random mating. In addition such studies can provide estimates of each karyotype's relative mating success, by comparing the frequencies of each karyotype in mating pairs with the

frequencies in the population at large. The common result of this kind of study on *D. pseudoobscura*, both in the laboratory and in the wild, is that although there can be large differences in mating success between male karyotypes, mating pattern with respect to female karyotype does not deviate significantly from random (Ehrman, 1967; Anderson *et al*, 1979; Salceda & Anderson, 1988). In fact variation in the efficiency with which male karyotypes mate has been reported for several *Drosophila* species (reviewed by Singh & Chatterjee, 1986). On the other hand non-random mating associated with karyotype appears only to have been documented for positively assortative mating in *D. melanogaster* (Stalker, 1976).

The experiments presented here are concerned with the reasons behind the persistence of one well documented inversion polymorphism. This is the polmorphism for the inversion on the right arm of the third chromosome, abbreviated *In(3R)P*, which is seen worldwide in *D. melanogaster* populations. Inbred lines were utilised where chromosomal heterosis may be more strongly expressed than in outbred populations. This may be expected as a result of different deleterious recessive mutations accumulating on the inverted and complementary standard (non-inverted) sequences. In the near absence of recombination between the inverted and standard arrangements Muller's ratchet should operate within each sequence. As well as this, recent work has shown that deleterious mutations may accumulate by simple drift, as selection lowers genetic diversity (Charlesworth, 1994). Early work on *D. subobscura* populations (reviewed by Krimbas, 1992) and recent studies of *D. melanogaster* (Inoue & Watanabe, 1992: see discussion) support this proposition. By comparing the severity of the heterosis present in inbred populations with that found in relatively outbred populations (also with *In(3R)P* segregating), it should be possible to identify the extent to which chromosomal heterosis has developed (see Chapter 5).

Certain studies of *D. melanogaster* have shown that the opportunity for mate choice results in more viable (at least for the component measured) offspring than those from random matings (Partridge, 1980; Taylor *et al*, 1987). It is therefore possible that mate choice may utilise non-additive effects on fitness such as those associated with inversion karyotype (as allowed by good genes models of sexual selection). Certainly, populations of the seaweed fly *Coelopa frigida* have been shown to exhibit mate preference for polymorphic inversions known to be associated with differences in developmental time, adult size, adult longevity and larval survival (Crocker & Day, 1987).

There have been many suggestions as to how such preferences could have evolved. However two types of model for the evolution of female preferences have recieved most attention (see the review by Kirkpatrick & Ryan, 1991). The first type, 'runaway process' or Fisherian models, depend on a genetic correlation between a preferred male trait and a female preference. The response of the preference to the evolution of the trait causes an unstable feedback loop which results in a runaway process capable of rapidly exaggerating the trait until it is maladaptive in relation to male survival.

The second type of model, 'good genes' or viability indicator models, invoke female preference for mates possessing a trait which indicates a genetic constitution that enhances viability. A female exercising such a preference passes genes for higher viability on to her offspring and thereby gains an evolutionary advantage. Consequently a genetic correlation is established between the viability enhancing genes and those for the preference.

The aforementioned work on *C. frigida* has more recently been extended to investigate the process by which females have evolved their mating preferences. A genetic correlation between the female

preference and the male trait has been discovered. To simplify somewhat, this acts such that females with the inversion karyotype associated with large male size exhibit a preference for large males. As the female preferences were not entirely consistent with the females mating to increase the fitness of their progeny, it seems likely that a Fisherian process may be responsible for the preferences. However, the possibility that a viability indicator process or indeed direct selection on discriminating females may also have been involved has not been discounted (Gilburn *et al*, 1992). Studies on *Cyrtodiopsis dalmanni*, the stalk-eyed fly (Wilkinson, 1993) and on *Poecilia reticulata*, the guppy (Houde, 1994) also indicate correlations between male traits and female mating preferences. However, work on these species has not yet reached the point where the processes leading to the evolution of the preferences can be identified.

One diagnostic test of whether a Fisherian or viability indicator process has given rise to a female preference is therefore whether it favours males that will endow their progeny with low viability. Only the Fisherian process should give rise to a preference which is maladaptive with respect to male and offspring viability. Consequently, as well as looking for evidence of heterosis the experiments presented here examined the possibility that mating was nonrandom with respect to *In(3R)P* karyotype. Any nonrandom mating pattern found could then be compared with that expected if females were mating so as to maximise the viability of their offspring.

Materials and Methods

The flies used in these experiments were derived from females collected from a wild population in March 1982 at the Furnace Creek date grove in Death Valley, California. This population was found to contain standard gene arrangements (ST) and three cosmopolitan

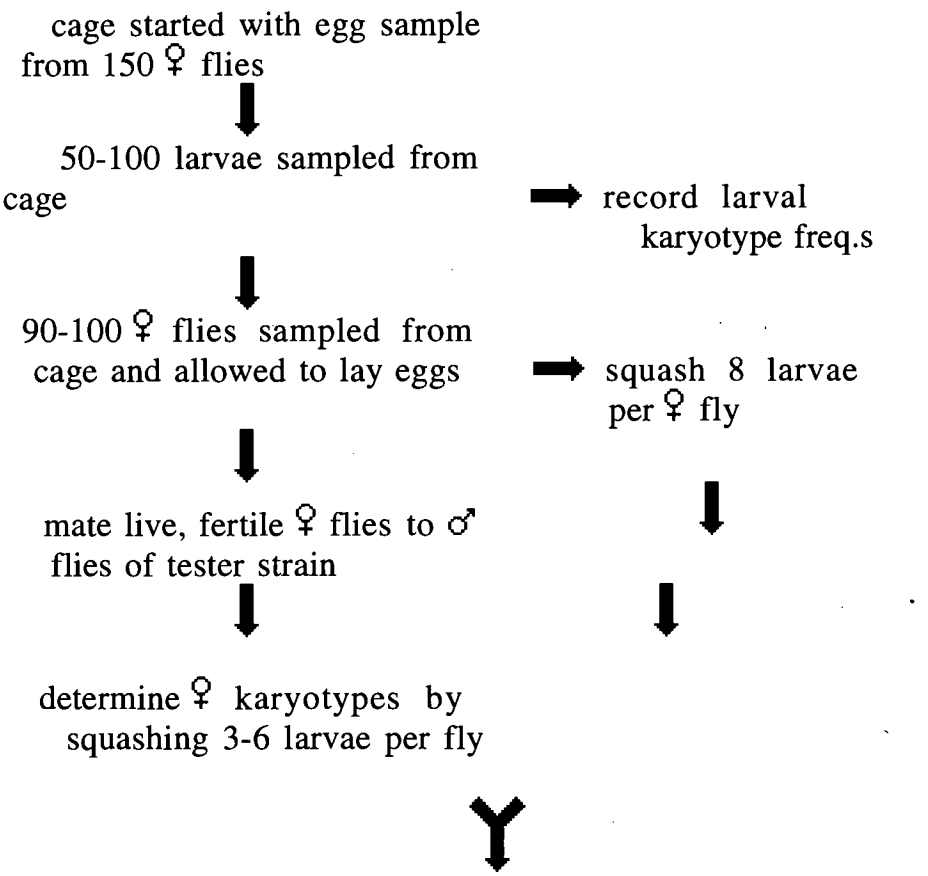
inversions at the following frequencies: *In(2L)t*, 0.105; *In(2R)NS*, 0.053; and *In(3R)P*, 0.456 (Ferrari, 1987). The several hundred Furnace Creek isofemale lines were then used in a crossing scheme to produce ninety lines homozygous for the second and third chromosomes, with their *X* and *Y* chromosomes derived from a balancer stock. However on examining the salivary gland chromosomes of larvae from the lines at Edinburgh in November, 1991 it was found that the lines were no longer homozygous for their second and third chromosomes. Presumably some contamination occurred during the period between November 1991 and March 1982. In the absence of access to any other lines containing the inversion there was no choice but to use these lines despite their dubious past. Both the lines that were used in this study were polymorphic for *In(3R)P* (Fig. 1, Appendix 4) but no other inversions. Despite the apparent contamination it was assumed that considerable inbreeding had occurred in both lines due to mating between sibs since capture. Both of these lines were maintained in vials containing standard *Drosophila* medium at 25°C on a 12 hour light and dark cycle in California (Ferranti, 1987). On their arrival at Edinburgh the flies were maintained on a similar sucrose-yeast medium (Trevitt & Partridge, 1991) at 25°C on the same light cycle.

All larvae were dissected alive in 0.7% NaCl saline, the extracted salivary glands were then fixed in a 1:1 solution of glacial acetic acid (45%) and ethanol (95%). Staining was done using freshly filtered 2% aceto-orcein solution, prepared by dissolving 2% synthetic orcein (Sigma Chemical Company, St. Louis, U. S. A.) in a 1:1 mixture of glacial acetic acid (45%) and lactic acid (85%) and refluxing for approximately 3 hours. All photomicrographs were taken using the PM-10AD Olympus Photomicrographic System.

Experimental Design

Each of the five replicate population cages was initiated by an egg sample laid in six milk bottles over 24 hrs by 150 female flies randomly selected from the two isofemale lines. The cage populations were kept at 25°C and provided with three milk bottles containing standard sucrose-yeast *Drosophila* medium every 14 days after initiation. The cage populations were also subject to a 12 hour light and dark cycle.

The experimental design can be summarised by the following diagram:



An egg sample was taken 21 days after initiation and 50-100 larval polytene squashes were prepared from it. Larval karyotype frequencies were recorded. A day later 90-100 females were sampled from the cage and allowed to lay eggs in individual vials for between 48 and 72 hours. (The same number of adult males were sampled from Cages 4 and 5 at the same time.) After this time the vials contained between 20 and 40 larvae resulting in very high survival (Partridge, personal communication). Eight of the larvae from each vial were then used to prepare polytene chromosome slides which were preserved.

The remaining fertile females were then mated to males of a tester strain (*In(2L)wg^P / In(2LR)Gla, Gla*) carrying two second chromosome inversions (Fig. 2, Appendix 4). Both of these inversions are X ray induced; *In(2LR)Gla* consists of *In(2LR)27D; 51E* superimposed on *In(2L)22D1-E1; 33F4-34A9* and *In(2L)wg^P* is a synonym for *In(2L)28A1-3; 32E-F* (for further information see Lindsley & Grell (1967)). Between 3 and 6 larvae (again grown at optimum densities) carrying either of these inversions were then squashed to determine the female's karyotype. In the two cages from which adult males were sampled each male was mated to a virgin tester strain female to determine the male's karyotype.

The karyotype of each female's mate was deduced by comparing her karyotype with that of her 8 original progeny. Deviations from random mating were then sought by comparing the inferred mating success of each adult male karyotype with its frequency. On the very rare occasions that progeny from a mating were found to have karyotypes that were inconsistent with the female's karyotype (inv/inv offspring from a st/st female for example), the mating was omitted from the data. Such occasions could conceivably have been the result of contamination, but it seems likely that scoring errors on the part of the experimenter were to blame (see below). Matings involving heterokaryotype females where only inv/st progeny were

found, presumably due to sampling error (see below), were also omitted from the data. Such omissions accounted for about 5% of matings.

There were two main sources of error associated with this experimental design. Firstly, sperm mixing due to multiple mating would have caused inaccuracy in the results. It is generally found that in excess of 90% of a twice-mated female's progeny are sired by the second male mate (Ashburner, 1989 Chap. 8). This assertion is in agreement with observations made in the present study while karyotyping adult females, where progeny lacking the second chromosome inversion markers of the tester strain were extremely rare. For instance, in a sample of 71 females collected from a cage set up identically to those in this experiment only 2 (or 2.82%) had a mixture of marked and unmarked progeny. In both cases the karyotypes of the unmarked progeny were consistent with having been produced in the first mating, between the female and the inferred male. In any case, of all the possible combinations of first and second males, the only ones that would have caused errors would have resulted in an overestimate of matings by heterokaryotypic males. This type of error would therefore have biased the results in the wrong direction to explain the large advantage to standard homokaryotype males observed in the raw, uncorrected (see next paragraph) data on mating success.

The second main source of inaccuracy was bias due to the failure to infer correct paternity because of the finite number of larvae karyotyped from each adult female(i.e. sampling error), exacerbated by viability differences. The probability that either of a male's third chromosomes were present in one of his third instar progeny is 0.5 (assuming a mating between inv/st and st/st individuals and no selection between the zygotic and third instar stages), as was the probability that it was not present. Therefore the probability of not sampling one of the male's chromosomes in my sample of 8 larvae

would have been 0.5^8=0.0039 per chromosome, or 0.0078 per male. However this problem is greater for matings between inv/st individuals (who as we shall see constituted the majority in the cages) where there is an increased risk of not sampling one or other homokaryotype. In addition the experiments documented here show that there was strong viability selection between zygote and adult stages in the cages, such that heterokaryotypic larvae survived relatively better. This means that it is quite plausible that homokaryotype progeny from a mating between heterokaryotypes were not sampled. This would be expected to lead to spuriously high inferred numbers of homokaryotypic male mates. This would have arisen when heterokaryotype progeny were found with representatives of only one of the homokaryotypes. In these cases a heterokaryotype male may be mistaken for a homokaryotype. In view of this problem a procedure for correcting the inferred mating data was developed (see Appendix 1).

Errors made on the part of the experimenter could also have affected the results. The quality of polytene chromosome preparations varied according to the amount of salivary gland tissue squashed amongst other factors, so some larvae may have been misclassified with respect to their karyotypes. This problem was most likely to have manifested itself as the misclassification of the inversion homokaryotype as the standard homokaryotype, and *vice versa*. This is because the differences between the two homokaryotypes were rather subtle in poorer salivary gland preparations, as distinguishing between them relied on chromosome banding differences. There was no reason to assume that one homokaryotype was misclassified more than the other. However a small experiment was undertaken in an effort to discover any errors in classification producing a consistent bias in the results.

Virgin females and males were collected from six milk bottles which contained an egg sample laid in the same way as those used to

initiate cages. Each female collected was placed, with one male, in a vial containing *Drosophila* medium. Eight of the progeny of 49 of the vials were dissected at the third instar larval stage and used to make polytene chromosome preparations which were mounted on microscope slides and made permanent. Both of the parents from these 49 vials were then mated to the tester strain *In(2L)wg^P / In(2LR)Gla, Gla* ,as described above, to determine their karyotypes. Given the parental karyotypes of each mating one can then ask whether the proportions of the karyotypes present in the 8 progeny from each vial conform to Hardy-Weinberg expectations. The 49 matings are shown in Table 2. Each of the mating catagories involving heterokaryotype females were G-tested to discover whether the ratios of karyotypes in the progeny were significantly different from those expected. As can be seen from Table 2.1, in each case there was no significant difference. This suggests that errors made by the experimenter were not sufficient to bias the results of karyotyping. These results also argue against any segregation distortion being associated with the inversion.

Results

Similarity between cages

From the karyotypic counts in the larvae, which were almost identical, the inversion was at a frequency of between 0.3 and 0.4 (Cage 1: 0.40; Cage 2: 0.43; Cage 3: 0.37; Cage 4: 0.35; Cage 5: 0.43) in all the cages. Two G-tests were done to look at variation in the data for larval and adult counts between the five cages. There were no significant differences between the larval samples (G=6.74 with 8df). The adult counts differed significantly (G=16.49 with 8df, $p<0.05$) due to the larval to adult viability heterosis being less severe in Cage 5: omitting Cage 5 from the test gave a nonsignificant G-value (G=8.74 with 6df). Cage 3 suffered bacterial infestation which appeared to have intensified the larval to adult viability heterosis associated with *In(3R)P*, but this effect was not significant.

Superficially then the cages seemed to be reasonable replicates.

Various G-tests were done on the data from each cage, as well as on the data set formed by pooling the results of all cages. The first G-test determined whether or not the observed larval counts differed from Hardy-Weinberg (H-W) expectations. In the first three cages the larval karyotype counts did not depart significantly from H-W equilibrium (Cage 1: G=3.22; Cage 2: G=1.70; Cage 3: G=1.30; all with 1df), most probably because the sample sizes were too small. When the larval sample size was increased, as in the case of the last cage, then there is a significant departure (G=6.63 with 1df, $p<0.05$) which is attributable to an excess of heterokaryotype (inv/st) individuals. The pooled larval sample from all the cages showed the same trend and also differed significantly from H-W equilibrium (G=23.56 with 1df, $p<0.001$).

The data were then tested to see whether larval and adult samples were significantly different in terms of the numbers of each genotype present (see Table 2.13 G-tests). In every cage except Cage 5 there was a significant difference ($p<0.05$). There appeared to be a strong heterotic effect of the inversion in larval to adult viability which results in a deficit of both homokaryotypes but especially the noninverted homokaryotype (st/st). This effect also appeared in the data pooled across all cages ($p<0.001$). As mentioned already, in Cage 5 the heterosis was less severe in the adult male counts to the extent that the difference between larval and adult counts in this cage failed to be significant. Cage 5 also differed from the other cages in terms of the trend it showed: an advantage to the inverted homokaryotype (inv/inv). However this trend was replaced by approximate equality between inv/st and inv/inv viabilities when the Cage 5 data pooled with Cage 4 (the only other cage from which adult males and females were sampled) data was tested.

Table 2: Matings set up to measure experimental error.

	♀ ST/ST	♀ INV/ST	♀ INV/INV
♂ ST/ST	1	7	0
♂ INV/ST	1	33	2
♂ INV/INV	0	5	0

Table 2.1: Progeny derived from experimental error matings pooled for each male mate karyotype. Progeny counts expected assuming unbiased segregation of inversion and perfect karyotyping are shown in parentheses.

♀ INV/ST mate	Pooled Progeny			G (2 df)
	ST/ST	INV/ST	INV/INV	
♂ ST/ST	25(28)	31(28)	0(0)	0.32
♂ INV/ST	74(66)	30(32)	60(66)	0.74
♂INV/INV	0(0)	22(20)	18(20)	0.20

Raw Data

Table 2.2: The karyotypic counts at third instar larval and adult stages are shown for each of 5 replicate cages. The standard (non-inverted) third chromosome homozygote is denoted ST/ST, the *In(3R)P* homozygote is denoted INV/INV and the heterozygote INV/ST.

Cage	Larvae			Adults		
no.	ST/ST	INV/ST	INV/INV	ST/ST	INV/ST	INV/INV
1	15	30	5	1	35	4
2	14	29	7	3	35	3
3	18	27	5	0	27	0
4	18	30	3	3	50	2
5	20	63	7	7	49	9
totals	85	179	27	14	196	18
%	29	62	9	6	86	8

Table 2.3: Data for matings inferred from Cages 1-5. Corrected data (see Appendix 1) are shown in parentheses.

Cage	Mating	♀ ST/ST	♀ INV/ST	♀ INV/INV
1	♂ ST/ST	0 (0)	19.62 (20)	0.94 (1)
2		1 (1)	28 (27.21)	0 (0)
3		0 (0)	8 (0)	0 (0)
4		0 (0)	13 (0.07)	0 (0)
5		0 (0)	10 (6.35)	0 (0)
1	♂ INV/ST	1 (1)	15.09 (15)	2.06 (2)
2		2 (2)	4 (5.39)	2 (2)
3		0 (0)	14 (22.62)	0 (0)
4		1 (1)	14 (27.13)	0 (0)
5		0 (0)	13 (17.21)	2 (2)
1	♂ INV/INV	0 (0)	1 (0.29)	0 (0)
2		0 (0)	2 (1.40)	0 (0)
3		0 (0)	3 (2.38)	0 (0)
4		0 (0)	2 (1.80)	0 (0)
5		0 (0)	2 (1.44)	0 (0)

Evidence for heterosis and non-random mating

Another G-test was done on the data relating to the frequency of matings in each cage. The expected 'random' values were calculated by determining the probability of each possible pairing of karyotypes (according to the adult counts) multiplied by the total number of matings 'observed' (that is inferred from adult females' progeny). In Cages 1 and 2 the random expected values were calculated in two ways: by assuming that adult male and female karyotypic frequencies were the same, and by using the pooled adult male frequencies from Cages 4 and 5 for the following reason. Adult male samples were only taken from Cages 4 and 5 and the results were equivocal. The male and female adult counts in Cage 4 were found not to differ significantly. However in Cage 5 the difference between male and female adult karyotype frequencies was significant ($G=9.08$ with 2df, $p<0.05$). This was attributable to the fact there were more st/st individuals in the male than in the female sample. This difference between males and females was not significant at the $p<0.05$ level ($G=8.26$ with 4df, $p<0.10$) when the data from Cages 4 and 5 are pooled together. Similarly the overall adult karyotype counts in the last two cages were not significantly different ($G=5.64$ with 2df, $p<0.10$). However, as a precaution against this level of difference between adult male and female counts the two sets of expected matings were calculated for Cages 1 and 2. In the same way two sets of expected results were calculated for the pooled data: one assuming male and female counts were equal in Cages 1, 2 and 3 and another assuming that the male counts were the same as those in Cages 4 and 5. Hence the double entries for Cages 1 and 2 and the pooled data in Table 2.5.

It proved difficult to calculate useful (i.e. non-zero) expected values for the purpose of investigating mating in Cage 3 because of insufficient data on adult frequencies: no homokaryotype adults were found. Instead the observed frequencies of male matings for

Table 2.4: The corrected number of matings (pooled across cages) observed for each possible mating between the three karyotypes. The numbers expected if mating occurred at random are given in brackets.

	♀ ST/ST	♀ INV/ST	♀ INV/INV
♂ ST/ST	0.97 (0.77)	58.61 (22.77)	0.78 (1.08)
♂INV/ST	4.03 (3.72)	82.44 (110.05)	6.22 (5.21)
♂INV/INV	0 (0.51)	6.95 (15.18)	0 (0.72)

Table 2.5: Summary of results of test for non-random mating (see text for explanation).

Cage no. (exp)	G value	df	p
1 (♂ =4+5)	38.16	6	< 0.001
1 (♂=♀)	105.95	6	< 0.001
2 (♂ =4+5)	72.37	6	< 0.001
2 (♂=♀)	107.32	6	< 0.001
3	9.74	2	< 0.01
4	4.43	6	> 0.05
5	2.51	6	> 0.05
pooled (♂ =4+5)	70.87	6	< 0.001
pooled (♂=♀)	128.41	6	< 0.001

each karyotype were compared to the observed adult female frequencies in a straightforward contingency G-test. In cages 1, 2 and 3 the pattern of mating was found to depart significantly from that expected if pairing was at random (Table 2.5). This was true regardless of whether adult male and female frequencies were assumed to be the same or the male frequencies of Cages 4 and 5 were assumed. This was also true of the mating pattern seen in the pooled data. As it appears from the pooled data in Table 2.4, this effect was mainly attributable to the fact that st/st males obtained more matings with inv/st females than expected, largely at the expense of inv/st males. This trend was seen in every cage (although it was not significant in Cage 5) except one and in the pooled data for all cages. The exception was Cage 4 where there was an excess of matings involving inv/st males and females, but this was not significant.

Next the randomness of mating for each karyotype and sex was tested for the pooled data: inv/st females ($p<0.001$ with either of the two sets of expected mating frequencies mentioned) and st/st males ($p<0.001$) were the only flies found to be mating nonrandomly. Again, the only exception was Cage 4 where the inv/st females were the only individuals found to mate non-randomly but this was due to an excess of matings with inv/st males ($G=7.26$ with 2 df, $p<0.05$). It should be noted however that the sample sizes for matings involving homokaryotype females were too small to detect any mating success differences for male karyotypes even in the pooled data.

Three-way analyses

A three-way table analysis was performed on the mating data pooled from both cages (Table 2.6) in order to discover whether the cage the flies occupied had any effect on mating frequencies. Further evidence could also be sought on whether male karyotype

Three-way analyses of G

Table 2.6: Three-way table constructed to examine interactions between female karyotype, cage number and male karyotype.

Female karyotype	Cage no.	Male karyotype			Totals
		♂ ST/ST	♂ INV/ST	♂ INV/INV	
♀ ST/ST	1	0	1	0	1
	2	1	2	0	3
	3	0	0	0	0
	4	0	1	0	1
	5	0	0	0	0
	subtotal	1	4	0	5
♀ INV/ST	1	20	15	0	35
	2	27	5.5	1.5	34
	3	0	23	2	25
	4	0	27	2	29
	5	6.5	17	1.5	25
	subtotal	53.5	87.5	7	148
♀ INV/INV	1	1	2	0	3
	2	0	2	0	2
	3	0	0	0	0
	4	0	0	0	0
	5	0	2	0	2
	subtotal	1	6	0	7
Totals		55.5	97.5	7	160

was a good predictor of female karyotype in the mating data. A series of hierarchical models were tested, investigating the three-factor and then various two-factor effects (using the computational procedure given in Sokal & Rohlf, 1981, Chap 17, p750).

The first test was for the three-factor interaction term (female karyotype by male karyotype by cage): a model which has this interaction term deleted is fitted to the data. The expected frequencies are calculated using a number of iterations of an iterative proportional fitting algorithim. Unfortunately it was not possible to get a G-value for this term because of deficiences in the data. (More specifically it was not possible to calculate expected frequencies with the column subtotals which add up to zero: female st/st with male inv/inv and female inv/inv with male inv/inv matings.) As a result the three-factor interaction term was dropped from the model. There are three possible models which have one two-factor effect deleted and these were used to test for the significance of two-factor effects.

Expected mating frequencies were then computed for the next model to be fitted: a model with the female karyotype by cage interaction term missing, in order to test the independence of female karyotype and cage given the male karyotype. A G-test for goodness of fit to this model was not significant. The female karyotype by male karyotype interaction term was the next to be deleted from the model, which also gave a nonsignificant G-value. The final two-factor interaction term investigated was male karyotype by cage and was significant ($p<0.001$). These results are given in Table 2.7. We must conclude therefore that the factors male karyotype and cage were not independent for each female karyotype. The Freeman-Tukey (F-T) deviates (which measure the degree of fit of each expected value to the observed) revealed that this effect was largely attributable to the difference in matings between inv/st females and st/st males across cages (see Table 2.8;

Table 2.7: Summary of the mating analysis examining two and three way interactions (explanation in text).

Interaction term	G value	df	deviates
♀ karyotype by ♂ karyotype by cage	-	16	-
♀ karyotype by cage	21.34	24	>
♀ karyotype by ♂ karyotype	13.32	20	>
♂ karyotype by cage	89.47	24	>

Table 2.8: Freeman-Tukey deviates for male karyotype by cage interaction, those exceeding the critical value are indicated by an asterix (explanation in text).

Female karyotype	Cage no.	Male Karyotype		
		♂ ST/ST	♂ INV/ST	♂ INV/INV
♀ ST/ST	1	-0.340	0.365	0.000
	2	0.570	-0.109	0.000
	3	0.000	0.000	0.000
	4	-0.342	0.365	0.000
	5	0.000	0.000	0.000
♀ INV/ST	1	1.871*	-1.280	-1.761*
	2	3.405*	-4.128*	0.080
	3	-5.095*	1.941*	0.753
	4	-5.552*	2.146*	0.599
	5	-0.807	0.612	0.412
♀ INV/INV	1	0.767	-0.213	0.000
	2	-0.464	0.343	0.000
	3	0.000	0.000	0.000
	4	0.000	0.000	0.000
	5	-0.464	0.343	0.000

the deviates exceeding the critical value 1.431 are indicated by asterixes). For Cages 1 and 2 the majority of matings were recorded in this category but none were present in the corrected data for Cages 3 and 4 where almost all of the matings were between inv/st flies. In summary then this three-way table analysis identified no consistent association between male and female karyotype across cages, and it underlines the differences in mating patterns between the cages.

Three three-way analyses were performed to test for associations between zygotic, larval and adult frequencies across the cages. An analysis examining such associations between zygotic and larval frequencies and the cage occupied revealed no significant interactions. Whereas the analysis for larval and adult frequencies and cage occupied identified three significant forms of interaction (see Table 2.9). The three-way interaction term was significant ($p<0.05$): the degree of association between larval and adult counts differed for different cages. The F-T deviates indicate that this was due to differences between Cages 3 and 5 in the frequencies of homokaryotype adults, that is in the severity of larval to adult heterosis. Surprisingly a significant G-value ($p<0.05$) was also obtained for the count type (larval or adult) and cage interaction. So whether the type of data was larval or adult was predicted by the cage occupied for each karyotype. This effect was attributable (from the F-T deviates) to reciprocal differences in larval and adult frequencies, between Cages 3 and 4 and Cage 5. It seems there were more inv/st adults in Cage 5 than in Cage 4, and more inv/st larvae in Cage 4 than in Cage 5. Similarly there were more st/st adults in Cage 5 than in Cage 3 and more st/st larvae in Cage 3 than in Cage 5. The fact that this interaction term was significant seems therefore to be explained by the reduced severity of heterosis in Cage 5 already discussed, rather than a more general phenomenon. Although the karyotypic frequency and cage interaction F-T deviates reflected the familiar difference in homokaryotypic adult

Table 2.9: Summary of the analysis examining two and three way interactions between larval and adult counts, karyotypic frequencies and cage (explanation in text).

Interaction term	G value	df	deviates
count type by karyo freq by cage	18.23	8	>
count type by cage	23.55	12	>
karyo freq by cage	23.23	16	>
karyo freq by count type	69.51	10	>

Table 2.10: Summary of the analysis examining two and three way interactions between zygotic and adult counts, karyotypic frequencies and cage (explanation in text).

Interaction term	G value	df	deviates
count type by karyo freq by cage	17.77	8	>
count type by cage	25.96	12	>
karyo freq by cage	26.19	16	>
karyo freq by count type	216.08	10	>

frequencies between Cages 3 and 5, this interaction failed to be significant. This reinforced earlier assertions that overall the five cages were replicates of each other. As expected the data type and karyotypic frequency interaction ($p<0.001$) accounted for most variation in the model as a result of heterosis. The depletive differences between the larval and adult data seemed most acute for the st/st karyotype, which was also in agreement with earlier findings.

Table 2.10 shows the results of the three-way analysis for zygotic and adult frequencies over the five cages also revealed three significant forms of association. As with the larval and adult analysis, the three-way interaction was significant ($p<0.05$) and again this was indicative of the differences in the strength of heterosis seen between Cages 3 and 5. Also the count type with cage interaction term was again significant ($p<0.05$) which this time was because of reciprocal differences between Cages 1 and 3 and Cage 5. In Cages 1 and 3 there are more st/st zygotes than adults, but in Cage 5 there are more st/st adults than zygotes. The cage with karyotypic frequency association term was again not significant but this time only narrowly so ($p<0.10$): the F-T deviates indicated differences in the strength of heterosis between Cages 3 and 5. Finally the karyotypic frequency by count type association was the most important interaction in the data ($p<0.001$) with all F-T deviates showing the same trend: a depletion of homokaryotype frequencies and an increase in inv/st frequencies between zygotic and adult stages. There were no significant differences in zygotic to adult viability between cages ($G=1.85$ with 6df).

Fitness estimates

For each cage the total relative viabilities (Table 2.15) of the three karyotypes were determined by inferring the zygotes which would be expected to result from the pattern of matings observed in the

cage, then comparing these values with the adult numbers (see Appendix 2). This provided a G-value for the difference in karyotype frequencies between these two life stages. The data from each cage was also used analogously to determine the differences in fitness among karyotypes between zygotic and third instar larval stages and between third instar larval stage and adulthood (Tables 2.12 and 2.13). The fitness component 'zygote-larval viability' confounds several different factors. Differences in karyotypic frequencies between zygotes (inferred from mating frequencies) and larvae could have been caused by differences in females' ability to form zygotes or to construct and lay viable eggs from these zygotes (i.e. female fecundity) as well as by differences in males' ability to fertilise eggs (i.e. sperm competition). Differences between karyotypes in viability as eggs and larvae could also have been involved. It was also thought helpful to pool the data from Cages 4 and 5 to check that the same fitness relationships between karyotypes identified in the pooled data were found when adult male frequencies were sampled.

Zygotic to larval viability was also estimated from progeny segregation ratios in the larvae that were housed in vials, for the purpose of correcting the inferred karyotype of the males that sired them (see Appendix 1). These estimates were accompanied by confidence limits (Table 2.11). A method to calculate comparable confidence limits for cage estimates was developed (see Appendix 2). This facilitated the comparison of cage estimates with vial estimates. If there had been intensified larval competition in the cages then one would expect cage and vial 95% confidence intervals not to overlap. Inspection of Tables 2.11 and 2.12 reveals that there is always overlap which lends weight to the proposition that levels of larval competition in the cages were similar to those in vials. It also suggests that there were no differences between karyotypes for female fecundity and the other factors confounded by this fitness estimate (see above).

Table 2.11: The relative zygotic to larval viability (including female fecundity) estimates for vials at approximately optimum density. All measures of significance are derived from differences in likelihood (see Appendix 1). The 95% confidence limits are given in parentheses.

Cage no.	Zygotic-larval viability			ΔL	df	p
	ST/ST	INV/ST	INV/INV			
1	1.09 (0.85,1.46)	1	0.61 (0.31,1.19)	1.734	2	> 0.05
2	0.83 (0.62,1.15)	1	0.53 (0.25,1.15)	3.335	2	< 0.05
3	1.42 (0.87,2.15)	1	0.44 (0.26,0.80)	5.558	2	< 0.01
4	0.96 (0.56,1.50)	1	0.80 (0.50,1.28)	0.769	2	> 0.05
5	1.41 (0.87,2.26)	1	0.57 (0.35,1.08)	2.824	2	> 0.05
pooled	1.14 (0.96,1.38)	1	0.49 (0.37,0.67)	14.402	2	< 0.001

Table 2.12: The relative zygotic to larval viability (including female fecundity) estimates for Cages 1-5. All measures of significance are G-tests (see text). The 95% confidence limits are given in parentheses (see Appendix 2).

Cage no.	Zygotic-larval viability			G	df	p
	ST/ST	INV/ST	INV/INV			
1	0.70 (0.32,3.28)	1	0.70 (0.18,2)	0.99	2	> 0.05
2	0.47 (0.25,1.32)	1	1.40 (0.37,3.76)	2.53	2	> 0.05
3	1.43 (0.66,2.54)	1	0.31 (0.15,0.91)	3.85	2	> 0.05
4	1.28 (0.64,2.59)	1	0.26 (0.08,0.66)	6.49	2	< 0.05
5	0.82 (0.41,1.47)	1	0.37 (0.13,0.85)	10.48	2	< 0.01
4 and 5	0.79 (0.49,1.25)	1	0.22 (0.06,0.50)	2.66	2	> 0.05
pooled	0.71 (0.51,0.98)	1	0.44 (0.28,0.70)	7.99	2	< 0.05

Table 2.13: The relative larval to adult viability estimates for Cages 1-5. (It was not possible to calculate meaningful estimates for Cage 3 because of insufficient data.) All measures of significance are G-tests (see text). The 95% confidence limits are given in parentheses (see Appendix 2).

Cage no.	Larval-adult viability			G	df	p
	ST/ST	INV/ST	INV/INV			
1	0.06 (0.01,0.41)	1	0.68 (0.12,2.08)	14.08	2	< 0.001
2	0.17 (0.07,0.61)	1	0.36 (0.02,1.24)	9.06	2	< 0.05
4	0.10 (0.04,0.39)	1	0.40 (0.05,1.58)	17.48	2	< 0.001
5	0.45 (0.16,1.18)	1	1.67 (0.50,3.89)	4.46	2	> 0.05
4 and 5	0.25 (0.11,0.58)	1	1.03 (0.37,1.32)	15.96	2	< 0.01
pooled	0.15 (0.08,0.29)	1	0.61 (0.41,0.89)	51.80	2	< 0.001

Perhaps surprisingly (in view of the many subcomponents it contains) cage estimates of zygote to larval viability (including fecundity) failed to be significant except in Cage 4 where there was an advantage to the st/st individuals (Table 2.12). No consistent trend such as heterosis was found across the cages. Increasing the larval sample size by pooling data for Cages 4 and 5 did give a significant inv/st advantage but this was not true of the inv/st advantage seen for the data pooled from all cages. There were no significant differences between the estimates for each cage for zygote to larval viability (G=3.46 with 8df).

A strong heterokaryotype advantage was shown for larval to adult viability in every cage except Cage 5 (Table 2.13) which showed a nonsignificant advantage to the inv/inv karyotype. This discrepancy between Cage 5 and the others was again attributable to the reduced severity of the heterosis in adult males from Cage 5. There were however no significant differences between the estimates for each cage (G=0.68 with 6df). The larval to adult viability estimates from the pooled data of Cages 4 and 5 showed a significant effect whereby inv/st had similar viability to inv/inv and the st/st karyotype suffered a disadvantage. This did not entirely contradict the data of the other cages where st/st was always found to be at the greatest disadvantage for this component of fitness. The data pooled across all cages showed significant larval to adult heterosis, with lowest viability for st/st.

There were also no significant differences between the estimates of total (i.e. zygotic to adult) viability and fecundity between cages (G=0.70 with 6df). Again strong heterosis was seen in every cage including non-significantly Cage 5 as well as in the pooled data, and again the st/st karyotype had lowest viability in the pooled data.

The relative mating success for each male karyotype was also calculated for each cage. The total number of matings which would

be expected for each karyotype at 'random' (as explained above) was compared with the corrected observed inferred matings (see Appendix 1). This calculation gives an estimate of the relative fitnesses of the three karyotypes, this time with respect to male mating success, and also a G-value indicating whether or not significant differences between the observed and expected matings exist. Unfortunately it was not possible to calculate female mating success as no independent samples of female adults were collected. Only the adult females sampled from the cages had their mates inferred, so that a comparison of adult female frequencies with the female mating frequencies data was uninformative.

The values for Cages 1, 2 and 3 shown in Table 2.14 were calculated assuming adult male frequencies were equal to those for the pooled data from Cages 4 and 5. Values of zero are misleading and reflect deficiences in the corrected mating numbers. All significant male mating success estimates showed an advantage to st/st males although the order of magnitude of this effect varied (Table 2.14). However there were no significant differences between the estimates for each cage (G=14.25 with 8df). In the data pooled from all cages there was roughly a three-fold advantage over inv/st males and an approximately five-fold advantage over inv/inv males.

Initially it was thought that the difference in male mating advantage between the last two cages may have given an estimate of the variation in mating advantage between cages. For example in Cage 4 inv/inv males constituted 4.17% of the population and had a 1.5-fold advantage over inv/st males. However in Cage 5 inv/inv males constituted 13.89% of the population and had a 2.59-fold disadvantage to inv/st males (and a 5.88-fold disadvantage to st/st males). On closer inspection however there was no evidence for negatively frequency-dependent inv/inv male mating success at the expense of inv/st males: neither male mating success indices

Table 2.14: The relative male mating success estimates for Cages 1-5. All measures of significance are G-tests. See text for explanation. The 95% confidence limits are given in parentheses (see Appendix 2).

Cage no.	♂ Mating Success			G	df	p
	ST/ST	INV/ST	INV/INV			
1	8.33	1	0	19.84	2	< 0.001
2	14.29	1	1.14	27.12	2	< 0.001
3	0	1	0.64	5.81	2	> 0.05
4	0	1	1.49	3.70	2	> 0.05
5	2.27 (0.37,3.27)	1	0.39 (0.03,1.58)	3.47	2	> 0.05
4 and 5	0.79 (0.25,1.06)	1	0.66 (0.12,1.87)	0.46	2	> 0.05
pooled	3.23 (1.43,4.11)	1	0.55 (0.19,0.89)	23.54	2	< 0.001

Table 2.15: The relative fitnesses in terms of total (zygotic-adult) viability (including female fecundity). (Cage 3 omitted: see text.) The 95% confidence limits are given in parentheses (see Appendix 2).

Cage no.	Total viability			G	df	p
	ST/ST	INV/ST	INV/INV			
1	0.04 (0.01,0.35)	1	0.48 (0.16,1.52)	21.18	2	< 0.001
2	0.10 (0.01,0.42)	1	0.52 (0.03,1.85)	15.45	2	< 0.001
4	0.12 (0.05,0.43)	1	0.08 (0.02,0.40)	17.86	2	< 0.001
5	0.37 (0.13,0.89)	1	0.60 (0.24,1.33)	3.11	2	> 0.05
4 and 5	0.20 (0.11,0.29)	1	0.23 (0.08,0.38)	19.56	2	< 0.001
pooled	0.11 (0.05,0.17)	1	0.27 (0.18,0.46)	65.04	2	< 0.001

Table 2.16: 'Adaptiveness' of mating (explanation in text).

Cage no.	Expected Mate	Observed Mate
1	inv/inv	st/st
2	inv/inv	st/st
3	st/st	inv/st
4	st/st	inv/inv
5	inv/inv	st/st
pooled	inv/inv	st/st

(G=3.86 with 2df) nor male karyotypic frequencies (G=3.64 with 2df) differed significantly between Cages 4 and 5. Similarly it was not possible to find evidence of positively frequency-dependent mating success for st/st males.

The likelihood estimates of male mating success differences (which compare the likelihood of the observed differences with that of no differences, see Appendix 2 for details) are likely to be more reliable than the G-testing results just mentioned. This is because the corrected mating data (see Appendix 1) are treated as observed data in a G-test, so that any 'noise' introduced by correction is not taken account of.

To maximise the viability of their offspring females should have mated with males of a particular karyotype. Most adult females were heterokaryotypic, which means they should have mated mainly with males belonging to the homokaryotype category with highest total viability: inv/inv males (Table 2.15). This would result in progeny which were half heterokaryotypes and half the most viable homokaryotypes. By this reasoning in every cage the pattern of mating seems to be maladaptive (Table 2.16). The zygotic to larval viability is used in Table 2.85 for Cage 3 in the absence of any other viability estimates for the cage.

Discussion

The main finding of these experiments has been a strong heterokaryotype advantage in larval to adult viability, such that 86% of the adults in the pooled data were heterokaryotypic. This study has also shown a mating advantage for st/st males relative to the other two male karyotypes (roughly a 3-fold advantage over inv/st males and almost a 6-fold advantage over inv/inv males). Despite suggestive trends no significant evidence of frequency-dependent male mating success was found. However, the small

sample sizes (for homokaryotypic males) imposed by heterosis and the inability to control adult male frequencies meant that this experimental design was badly suited to detecting such effects. The male mating success differences observed appeared to be unimportant (assuming the populations were at a stable equilibrium) in determining the dynamics of the polymorphism: they did not predict the strong heterosis seen in adult flies.

No evidence was found for effects of karyotype on female mating success (which includes factors such as fecundity, fertility and propensity to mate). One could speculate however (from the zygotic to larval viability estimates) that there could have been an effect of karyotype on a major component of female mating success, for example female fecundity. Again, it was a shortcoming of the experimental design that adult female samples, independent of the samples which had their mates' inferred, were not taken. Such samples would have allowed the estimation of female mating success which were not confounded by other factors.

The main body of work done previously which looked at karyotypic fitness components for the *In(3R)P* polymorphism was by Barnes (Barnes, 1983; Barnes & Merrell, 1985). This work had the added complication that the two populations studied had been selected for resistance to the pesticide DDT. Nevertheless the similarities between Barnes' work and this study are striking. Barnes (1983) showed that in the presence of DDT both populations reached an intermediate equilibrium condition of 0-10% st/st,70-80% inv/st, and 20-30% inv/inv. This heterokaryotype excess was mainly attributable to larval viability differences. In addition the standard chromosome was found to confer a male mating success advantage (attributable to female preference) on male carriers, as well as fecundity and fertility advantages on female carriers (Barnes & Merrell, 1985). Significant rare male mating advantages were found for st/st and inv/inv males. In one population under study it was

shown that this mating success advantage did predict the dynamics of the polymorphism (i.e. the inverted chromosome began to decrease in frequency), but only in the absence of DDT. The *In(3R)P* polymorphism had previously been reported as exhibiting frequency-dependent selection under 'normal' laboratory conditions, but this was balancing rather than directional selection (Nassar *et al*, 1973). Numerous other studies have identified adult fitness components and especially male mating success as the major components operating in natural populations (see introduction to this chapter).

To the extent that male mating success represents female mating preferences the females' preferences for males in this study would seem to have been maladaptive. That is these preferences would not have maximised the viability of the offspring produced. (This was also true of the Barnes and Merrell (1985) study, although the authors failed to mention it.) Thus the results were inconsistent with viability indicator models for the evolution of female preferences, but were consistent with Fisherian models. Had the Fisherian process been acting it would have had to operate through a correlation between the female preference and the preferred trait. In these experiments this could have been caused by linkage disequilibrium arising through non-random mating, with both preferred male trait and female preference located within the standard gene arrangement.

Brittnacher (1981) showed that the genetic load uncovered by making whole *D. pseudoobscura* and *D. melanogaster* chromosomes isogenic was larger for adult male mating success than for larval viability. Work done on *D. melanogaster* by Sharp (1984) also confirmed that inbreeding due to full-sib mating can result in a rapid decrease in male competitive ability. A similar phenomenon may have caused the apparent mating advantage to the standard chromosome in this study. One could imagine the situation where

inbreeding may have skewed balancing homokaryotypic mating advantages so that the inverted chromosome suffered a stronger effect of inbreeding than the standard chromosome. This effect may be expected as some inversions are persistently found in association with rather deleterious genes, and contain more than standard uninverted chromosomes (briefly reviewed by Lemeunier & Aulard, 1992). However this mating advantage need not cause the elimination of the inversion if strong heterosis for viability masks its effect. Heterosis could result from associative overdominance via the accumulation of deleterious recessives and then be exaggerated by traumatic environments. Such an environmental effect may explain the severity of heterosis seen in Cage 3 of this study (which suffered bacterial contamination) and in the DDT containing environment of the Barnes and Merrell (1985) study. In the latter study, cages were initiated with eight homokaryotypic lines for each arrangement, which may have led to inbreeding.

Inbreeding induced mating effects could also help to explain observations on the elimination of cosmopolitan inversions (including *In(3R)P*) from population cages initiated with flies from 40-600 inbred lines. Where these cages were initiated with only one or two inbred lines, producing a relatively more inbred population, the polymorphism is usually maintained (Inoue & Watanabe, 1992). One can speculate that some level of comparatively light inbreeding may cause a mating disadvantage for inverted chromosomes, as in the 40-600 lines cages. In such cages this effect could determine the fate of the inversion causing it to be removed from the population. However much heavier inbreeding (with fewer chromosomes present with which to recombine), as in the case of cages started with one or two lines, could maintain inversion polymorphism, due to chromosomal heterosis (as was suggested by these authors), despite mating patterns favouring the loss of the inversion.

Before comparing the heterotic and mating effects seen here with observations on a comparatively outbred population (see Chapter 5) it was thought necessary to verify the effects already seen with more data on male karyotypic frequencies. In addition it was of interest to sample cages containing these same inbred lines over several generations to test whether the situation discussed above is stable. These data are presented in the next chapter.

Chapter 3: The stability of the *In(3R)P* polymorphism in inbred lines over six generations: further evidence for heterosis and non-random mating

Laboratory populations of *Drosophila*, polymorphic for inversions, have been studied since the 1930s (Lemeunier & Aulard, 1992). Two main strategies have been utilised in constructing cage populations. Several seperate strains may be derived that have two or more gene arrangements on a chromosome and a standardised genetic background. This strategy was used in the early experiments of Dobzhansky and his associates (eg. Wright & Dobzhansky, 1946; Anderson *et al*, 1967) to investigate the polymorphism in *D. pseudoobscura*. The other strategy has been to cage a large population directly from the wild and observe its evolution. This method seems to have originated with Krimbas' (1967) work on *D. subobscura* inversion polymorphisms.

There are two common consequences of caging a *Drosophila* population polymorphic for an inversion which, as we shall see, may be related to the strategies mentioned above. Dobzhansky and Pavlovsky (1953) described the maintenance of inversion polymorphism in replicate populations started with F_1 hybrids between 12 standard and 12 inverted homokaryotype lines in *D. pseudoobscura*. The same equilibrium was reached in all four replicate cages after about 12 generations. In another experiment (designed to support 'founder effects') Dobzhansky and Pavlovsky (1957) started twenty cages with F_2 hybrids. These hybrids were the product of crosses between F_1 progeny which were in turn the product of crosses between 22 lines homokaryotypic for one of two gene arrangements. The result again was the maintenance of polymorphism regardless of the fact that ten cages were founded by 20 F_2 hybrids and ten by 4000 F_2 hybrids. Indeed many of Dobzhansky's experiments, which reached repeatable stable polymorphisms, were started in similar ways (Powell & Krimbas, 1992). Similar results have been reported for populations of *D. ananassae* established by mixing inbred homozygous lines (Tobari & Kojima, 1967; Kojima and Tobari, 1969).

In contrast Anderson *et al* (1967) reported the rapid increase of the standard gene arrangement in ten cages established with between 24 and 123 *D.pseudoobscura* isofemale lines. Inoue (1979) also showed that in natural *D. melanogaster* populations all common cosmopolitan inversions were rapidly eliminated in the laboratory. This work was extended to show that the number of isofemale lines used to initiate cages was the critical factor. Judging from the results of Dobzhansky and Pavlovsky (1957) and Anderson et al (1967) one could speculate that the critical number of lines required to maintain inversion polymorphism in *D. pseudoobscura* is less than 24. However in small vial populations or in cages started with one or two lines the inversions were kept at high levels: for example *In(3R)P* was reportedly maintained at an average frequency of 39.08% in this way (Inoue & Watanabe, 1992). Das and Singh (1990) have also described the maintenance of three inversions never before detected in a population of *D. melanogaster*. In fact the maintenance of inversions in isofemale lines is a common observation and has also been reported in *D. ananassae* (Singh, 1982) and *D. pavani* (Brncic, 1969).

In the experiments presented in this chapter the observations of Chapter 2 were largely confirmed. Strong viability heterosis appeared to maintain the polymorphism stably for six generations, and again seemed to act between larval and adult stages. On the other hand the male mating success advantage to carriers of the standard chromosome failed to reappear, although mating patterns were again non-random. The similarities and differences between these experiments and those of Chapter 2 are discussed further at the end of this chapter.

Materials and Methods

As in the experiments described in the last chapter the two cages were initiated by an egg sample laid over 24 hours by 150 female flies. These females were randomly selected from the

same two inbred isofemale lines polymorphic for the inversion. In this experiment two replicate cages were allowed to continue for about 6 generations; samples were taken every 21 days (about 2 generations) beginning 21 days after initiation. (Adult flies had been observed as emerging from their pupae 10 days after initiation in the experiments of the last chapter.) In total three samples were taken from each cage.

There was one change in the experimental design as compared with the last chapter. Additional samples of eggs were collected during each sampling episode in this experiment, with the intention of estimating egg to larval viability and female fecundity. Samples of about 100 eggs were laid in three or four vials placed in the cage. These eggs were then allowed to hatch and develop to the third instar larval stage at optimal densities with the intention of squashing and karyotyping all of the resulting larvae. Unfortunately in each sample 10-20% of the larvae managed to pupate before they could be karyotyped. Ignoring these pupae there were between 5-10% eggs which did not hatch.

Results

Similarity between the cages

The frequency of the inversion was, as in the last chapter's experiments, was around 0.3 to 0.4 in each sample (AI: 0.37; AII: 0.29; AIII: 0.34; BI: 0.46; BII: 0.41; BIII: 0.44). These frequencies were calculated from the larval counts and at first sight appeared to show a decline in the frequency of the inversion. However G-testing showed that in Cage A there were no significant differences between consecutive sample counts of eggs, larvae, adult males or adult females. This was also true of Cage B. The state of the cages with regard to the karyotypic frequencies at these life stages would therefore seem to have been stable. Also no significant differences were found between any sequentially equivalent samples from cages A and B (sample AI with sample BI etc), so superficially at least the cages were replicates. There

were also no significant differences between sequentially equivalent mating frequency samples between the cages. However there were significant differences between samples within each cage. In both cages this took the form of differences between the first sample and both the second and third samples, although the second and third samples were not significantly different from each other. In samples AII and AIII there were less matings between heterokaryotypes than there were in AI; this was because there were more matings between inv/st females and both homokaryotype males. The same trend was repeated in samples BII and BIII with respect to BI. There were no significant differences between the pooled mating frequencies for Cages A and B. Again, it seems appropriate to regard Cages A and B as replicates.

As was mentioned above, there was one difference between the methodology of these experiments and the ones presented in the last chapter: egg samples were included in each cage sample. The raw data for the egg samples are in Table 3. On first inspection these data seemed rather surprising: in each cage heterokaryotype eggs constituted around 55% of the sample. Assuming that the majority of matings should involve heterokaryotype females (see Table 3.1) it should have been impossible to get greater than 50% heterokaryotype offspring. Presumably many of the individuals who appeared in the data as mortalities or pupae are homokaryotypic. Possible explanations for such a heterokaryotype excess include differences between karyotypes in early (i.e. between zygote and egg stages) mortality or in the rates of development of karyotypes. The individuals that succeeded in pupating were those that pupated during the night over roughly a 48-72 hour period. It follows that there may have been variation in development time to the extent that larvae pupated 24 hours or more apart (since they were all laid in a roughly 3 hour period). However it is not known how many of the pupae that were observed successfully eclosed. Finally a heterozygote excess in the egg samples could be explained by disassortative mating between homokaryotypes

Table 3: Data for egg counts from Cages A and B.

Cage Sample	Eggs			Pupae	Mortality
	ST/ST	INV/ST	INV/INV		
AI	15	45	20	10	6
AII	20	51	18	14	8
AIII	20	46	22	9	7
pooled	55	142	60	33	21
BI	19	52	18	17	9
BII	20	42	15	16	5
BIII	16	38	20	19	7
pooled	55	132	53	52	21

Table 3.1: Data for larval and adult counts from Cages A and B.

Cage Sample	Larvae			Adults		
	ST/ST	INV/ST	INV/INV	ST/ST	INV/ST	INV/INV
AI	33	35	12	10	42	6
AII	46	43	6	10	39	7
AIII	46	34	15	14	42	8
pooled	125	112	33	34	123	21
BI	24	47	17	3	34	4
BII	32	52	15	6	42	9
BIII	21	59	10	13	42	15
pooled	77	158	42	22	118	28

Table 3.2: Data for matings from Cages A and B. Corrected matings shown in parentheses (see Appendix 1).

Cage Sample	Mating	♀ ST/ST	♀ INV/ST	♀ INV/INV
AI	♂ ST/ST	0 (0)	5 (0.11)	1 (0.97)
AII		1 (0.99)	7 (2.59)	1 (0)
AIII		0 (0)	5 (4.35)	0 (0)
BI		1 (0.99)	5 (0)	0 (0)
BII		0 (0)	5 (3.84)	1 (0.96)
BIII		2 (1.99)	7 (7.92)	0 (0)
AI	♂ INV/ST	4 (4)	17 (22.89)	2 (2.03)
AII		4 (7.11)	5 (7.25)	2 (5)
AIII		5 (5)	5 (6.27)	3 (3.01)
BI		2 (2.01)	11 (17.65)	2 (2)
BII		4 (4.02)	6 (7.58)	4 (4.04)
BIII		4 (4.01)	7 (8.88)	7 (7.04)
AI	♂ INV/INV	0 (0)	0 (0)	0 (0)
AII		0 (0.90)	5 (1.16)	0 (0)
AIII		0 (0)	6 (5.38)	1 (0.99)
BI		0 (0)	4 (0.35)	0 (0)
BII		1 (0.98)	6 (5.58)	0 (0)
BIII		0 (0)	5 (2.20)	2 (1.96)

or differences in fecundity between females of different karyotypes. In fact the heterokaryotype excess in the egg samples was never statistically significant (see Table 3.12), that is the egg frequencies were not significantly different from those zygotic frequencies one would predict from the observed matings. These egg samples may therefore indirectly provide evidence for a lack of disassortative mating between homokaryotypes as well as a lack of differences between karyotypes in fecundity, early mortality and rate of development. Alternatively such differences could have acted so as to cancel one another out and merely give the impression of constancy in karyotypic frequencies between zygotic and egg stages. In short the egg samples gave little additional information on selective differences between these stages and mating patterns in the cages.

Heterosis and non-random mating

Various G tests were carried out on the data from each cage to investigate any heterosis or departures from non-random mating associated with the inversion. First the larval karyotypic frequencies observed in each sample were compared with expected values assuming Hardy-Weinberg (H-W) equilibrium. In most samples there were no significant departures from H-W equilibrium despite, as we shall see, the existence of non-random mating. In the two samples where there were significant departures (the third sample in both cages) they occurred for different reasons. In sample AIII ($G=3.92$ with 1df, $p<0.05$) this was a result of an excess of both homokaryotypes, whereas the departure in sample BIII ($G=10.16$ with 1df, $p<0.01$) was attributable to an excess of heterokaryotypes. The pooled samples for each cage also departed significantly from H-W expectations (Cage A: $G=14.34$ with 1df, $p<0.001$; Cage B: $G=29.40$ with 1df, $p<0.001$) and the cause was the same in both cases. Both samples had lower numbers of st/st larvae and higher numbers of inv/st and inv/inv larvae than expected.

The karyotypic counts in the larvae were also compared with those in the adults (see Table 3.15). As in the last chapter's experiments there was evidence for a heterotic effect of the inversion in viability between third instar larval and adult stages. This was the case in every sample except BIII. There were no significant differences between adult male and female counts in samples from either cage.

The pattern of mating in each sample was compared with those expected under 'random' mating (see Tables 3.3 to 3.6). As in the last chapter the term 'random' refers to the numbers of matings expected if each karyotype had an equal chance of mating (and of laying the same number of eggs in females), so that its success depended only on its frequency in the adults. Although departures from random mating were detected they were not as substantial as in previous experiments. Significant departures were seen only in samples AI and AIII from Cage A. In AI this was chiefly attributable to a greater number of matings than expected involving only heterokaryotypes. In AIII on the other hand the effect was due to a greater number of inv/inv male and inv/st female matings and (to a lesser degree) inv/st male and st/st female matings than anticipated. Increases in matings between inv/st males and st/st females were also observed in sample AII but were significant only at the $p<0.10$ level. The pooled samples from Cage A also showed this trend but it failed to be significant.

Mating in Cage B was not significantly different from the random expectation. However, trends similar to those observed in Cage A were seen, albeit non-significantly. In BI (as in AI) there was an increase in matings between heterokaryotypes which contrasted with the patterns seen in samples BII and BIII. The BII sample was marginally non-significant at the $p<0.05$ level and the main trends were an increase in matings between inv/st males and st/st females (as in AII and AIII) and those between inv/inv males and inv/st females (as in AIII). The BIII sample and the pooled Cage B sample were also significant only at the $p<0.10$

Table 3.3: Corrected matings inferred for adult females from Cage A (the numbers expected if mating occurred at random are given in parenthesis).

	♀ ST/ST	♀ INV/ST	♀ INV/INV
♂ ST/ST	0.95 (4.15)	8.42 (11.54)	0.88 (2.77)
♂ INV/ST	16.12 (12.12)	36.34 (33.65)	10.13 (8.08)
♂ INV/INV	0.93 (1.73)	5.24 (4.81)	0.99 (1.15)

Table 3.4: Matings inferred for adult females from Cage B (the numbers expected if mating occurred at random are given in parenthesis).

	♀ ST/ST	♀ INV/ST	♀ INV/INV
♂ ST/ST	2.96 (1.18)	11.11 (4.55)	0.88 (1.35)
♂ INV/ST	10.08 (10.12)	34.47 (39.04)	13.14 (11.57)
♂ INV/INV	0.96 (2.70)	8.42 (10.41)	1.98 (3.08)

Table 3.5: Matings inferred for adult females from Cages A and B (the numbers expected if mating occurred at random are given in parenthesis).

	♀ ST/ST	♀ INV/ST	♀ INV/INV
♂ ST/ST	3.92 (4.31)	20.44 (19.89)	1.78 (2.98)
♂ INV/ST	26.21 (18.07)	70.49 (83.42)	23.25 (12.51)
♂ INV/INV	1.87 (3.61)	13.07 (16.68)	2.97 (2.50)

Table 3.6: Summary of results of test for non-random mating.

Cage sample	G value	df	p
AI	22.80	6	<0.001
AII	11.52	6	>0.05
AIII	14.10	6	<0.05
A pooled	7.67	6	>0.05
BI	4.58	6	>0.05
BII	12.47	6	>0.05
BIII	11.55	6	>0.05
B pooled	10.76	6	>0.05
A and B pooled	7.408	6	>0.05

Three-Way Table Analysis

Table 3.7: Summary of the analysis examining two and three way interactions in the egg and larval frequencies data (explanation in text).

Interaction	G	df	deviates
cage by count by karyo freqs	14.5	10	>
cage by count	17.32	15	>
cage by karyo freqs	35.31	20	>
count by karyo freqs	59.99	12	>

level and showed increases in matings between inv/st females and homokaryotype males as in sample AIII. This time however it was st/st males that achieved the increased matings, as opposed to inv/inv males in AIII. The data pooled for both Cages A and B did not show a significant departure from random mating. The trend however remained the same: higher numbers of matings than expected for those involving inv/st females and both homokaryotype males, but particularly the inv/inv males.

Three-way table analyses

A three-way table analysis was performed on the data pooled from both cages in order to discover whether the cage the flies occupied had any effect on mating frequencies. As well as this further evidence could be sought regarding whether male karyotype was a good predictor of female karyotype in the mating data. A series of hierarchical models were tested, investigating the three-factor and then various two-factor effects (the computational procedure was identical to that used in Chapter 2).

In a three-way table the first test is for the three-factor interaction (female karyotype by male karyotype by cage): a model which has this interaction term deleted is fitted to the data. The expected frequencies were calculated after four iterations of an iterative proportional fitting algorithim. Comparison of the expected and observed values using the G-test yielded an insignificant G-value (G=1.36 with 4df). In addition calculation of the Freeman-Tukey deviates (which measure the degree of fit of each expected value to the observed) revealed that none of them exceeded the rough criterion for being 'large' (see Sokal & Rohlf, 1981, Chap 17, p755). There was therefore no evidence of a three-factor interaction, so this term was dropped from the model. The G-values obtained by fitting models lacking the female karyotype by cage interaction term (G=2.86 with 6df), the female karyotype by male karyotype term (G=5.85 with 8df) and the male karyotype by cage term (G=2.44 with 6df) were all

non-significant. In each case the Freeman-Tukey (F-T) deviates were all less than the critical value mentioned above. It is safe to conclude that for a given female karyotype the matings they achieved with any male karyotype were unaffected by the cage they happened to occupy. Similarly, for a given male karyotype the matings they achieved with any female karyotype were unaffected by the cage they occupied. The fact that the female karyotype by male karyotype interaction was not significant lends further weight to the conclusion that there was no pervasive pattern of non-random mating.

Five other three-way table analyses were performed on the data relating to zygotic, egg, larval and adult frequencies. These analyses were intended to uncover any effect of the cage sample number on the samples, as well as to see whether karyotypic frequencies in one life stage affected the next. The data from the two cages were classified according to sample, data type (e.g. zygotic or egg data) and karyotype. A test of zygotic frequencies by egg frequencies by cage gave no significant G-values for the three-way or two-way interaction terms. The F-T deviates were all below their critical values. These results would be expected in the absence of selection between zygotic and egg stages i.e. no differences between karyotypes in female fecundity.

A similar test involving egg and larval data showed that there were significant interactions for two of the possible two-way interactions (Table 3.7). Cage sample appeared to be a good predictor of the karyotypic proportions observed whether the data was for eggs or larvae ($p<0.05$), which indicates heterogeneity between cage samples. This was a result of the differences between cages already discussed: in Cage A every sample had a significant advantage to the st/st individuals between these stages, but this advantage varied in magnitude. This combined with the fact that only one Cage B sample showed a significant effect which was heterotic meant that there was significant heterogeneity between samples. Also

data type was a good predictor of the karyotypic proportions observed ($p<0.001$) because of the st/st bias in the larval samples, this supports the idea of selection between egg and larval stages. The equivalent two-way interaction terms were also significant between larval and adult (Table 3.8) and zygotic and larval stages (Table 3.9). Karyotypic frequencies (larval or adult this time) were again dependent upon cage sample ($p<0.01$), and again this was presumably because of differences between the cages in the strength of heterosis. In Cage A two samples showed significant heterosis between larvae and adults but one had a significant inv/inv advantage. In Cage B one sample showed no significant effect and in the other two the heterosis was less severe than in Cage A. The karyotypic frequencies observed also depended heavily upon whether the data was for larvae or adults ($p=0.001$). Again this suggested differences between samples and implicated selection between larval and adult stages.

Analysis of this kind was also carried out for zygotic to larval viability (including female fecundity), again to allow comparison with experiments which did not involve egg sampling (Table 3.9). In common with egg to larval viability there was a significant ($p<0.05$) interaction between cage and karyotypic frequencies. Again this was a result of the variation in the strength of the st/st advantage in Cage A and in the heterosis in Cage B (to the point that it was non-existent in BII). In contrast with the egg to larval analysis, there was no significant interaction between data type and karyotypic frequencies, implying no persistent selection between the zygotic and larval stages. However examination of the F-T deviates revealed that certain samples (particularly AII, AIII and BIII) fit such a model rather badly. These are the samples which provide the strongest examples of the trends in either cage.

Zygotic to adult viability and female fecundity showed no significant interaction terms apart from the data type by

Table 3.8: Summary of the analysis examining two and three way interactions in the larval and adult frequencies data (explanation in text).

Interaction	G	df	deviates
cage by count by karyo freqs	17.20	10	>
cage by count	20.51	15	>
cage by karyo freqs	43.92	20	>
count by karyo freqs	170.66	12	>

Table 3.9: Summary of the analysis examining two and three way interactions in the zygotic and larval frequencies data (explanation in text).

Interaction	G	df	deviates
cage by count by karyo freqs	8.31	10	>
cage by count	11.51	15	>
cage by karyo freqs	33.36	20	>
count by karyo freqs	19.69	12	>

Table 3.10: Summary of the analysis examining two and three way interactions in the zygotic and adult frequencies data (explanation in text).

Interaction	G	df	deviates
cage by count by karyo freqs	3.86	10	<
cage by count	5.26	15	<
cage by karyo freqs	13.41	20	>
count by karyo freqs	64.59	12	>

karyotypic frequencies term ($p<0.001$; Table 3.10). Selection between these stages, leading to a deficit of homokaryotypic adults was responsible. This effect was particularly important in sample BI: the only sample which yielded a significant heterotic effect in earlier G-tests.

Fitness estimates

All fitness component estimates were calculated as in the experiments in the last chapter. The fitness component 'female fecundity' is something of an amalgam. Differences in karyotypic frequencies between projected zygotes and eggs could have been caused by differences in females' ability to form zygotes (or differences in the males' ability i.e. sperm competition) or to construct and lay viable eggs from these zygotes (i.e. female fecundity) as well as by differences between karyotypes in viability as eggs. In common with the raw data counts, fitness estimates for each component (Tables 3.11 to 3.17) revealed no significant differences between samples from Cages A and B. Again, this suggests the cages are replicates of each other and show stable polymorphisms. The component zygotic to larval viability (Table 3.14) appears to be unnecessary, as it overlaps with female fecundity and egg to larval viability measures. It was calculated because of the ambiguities in the egg data and to facilitate comparison with other experiments which did not include data on egg samples.

In these experiments viability estimates were broken down into the relative karyotypic viabilities over certain stages of the life cycle. As in the last chapter there were two kinds of estimates for zygotic to larval viability (including female fecundity), one for samples in vials (made to allow the correction of mating data) and one for cages. The estimate made for populations in vials (at near optimum density) shows significant heterosis in the samples pooled over Cage A and both A and B (Table 3.11). The effect seen is however very weak, with almost equal fitnesses for st/st and inv/st. The same two samples were the only ones to show significant differences between karyotypes for this component in the cages (Table 3.14). Rather than heterosis the effect seen here

Table 3.11: The relative zygotic to larval viability estimates (including female fecundity) for vials at approximately optimum density. All measures of significance are derived from differences in likelihood (see Appendix 1). The 95% confidence limits are given in parentheses.

Cage no.	Zygotic-larval viability			ΔL	df	p
	ST/ST	INV/ST	INV/INV			
AI	0.98 (0.66,1.43)	1	0.65 (0.40,1.06)	2.17	2	>0.05
AII	0.84 (0.54,1.38)	1	0.59 (0.34,1.07)	2.85	2	>0.05
AIII	0.92 (0.56,1.50)	1	0.88 (0.53,1.50)	0.26	2	>0.05
Apooled	0.88 (0.68,1.16)	1	0.76 (0.56,1.03)	3.32	2	<0.05
BI	0.96 (0.56,1.50)	1	0.80 (0.50,1.28)	0.77	2	>0.05
BII	1.11 (0.63,2.02)	1	0.79 (0.45,1.38)	2.07	2	>0.05
BIII	0.92 (0.60,1.43)	1	0.86 (0.51,1.40)	0.49	2	>0.05
Bpooled	1 (0.76,1.34)	1	0.85 (0.67,1.15)	0.93	2	>0.05
A+B	0.99 (0.77,1.15)	1	0.79 (0.65,0.99)	3.68	2	<0.05

was an advantage to the st/st karyotype, followed by inv/st and then inv/inv. This could indicate increased larval competition in the cages altering the karyotypic fitnesses seen at optimum densities. In Cage A there was some evidence of more intense selection than in the equivalent vial populations (see confidence limits for st/st karyotype in Tables 3.11 and 3.14). As the 95% confidence limits of the vial and cage fitnesses overlapped in all but this case there was little statistical support for such a difference in larval competition.

The component zygote to larval viability can be compared with those estimates made for karyotypic differences in female fecundity (Table 3.12) and in viability between egg and larval stages (Table 3.13). No significant differences were found in fecundity between karyotypes but there was a non-significant heterotic effect. This contrasts with the estimates for egg to larval viability, here the st/st karyotype had the highest viability, followed by inv/st and then inv/inv. This effect was seen in both cages and was significant in the data pooled across both cages. It therefore seems probable that any advantage to the st/st karyotype in zygotic to larval viability was reducible to selection occurring between the egg and larval stages.

As in the last chapter the strongest heterotic effect of the inversion was seen in larval to adult viability (Table 3.15). Significant effects were seen in all samples except BIII and all significant effects were of the kind seen in the last chapter (heterosis which seemed particularly disadvantageous for the st/st individuals) except in sample AII. In this sample st/st still had the lowest viability but the positions of the other two karyotypes were reversed, inv/inv having the highest viability. This provides no evidence of frequency-dependent viability however, as larval frequencies did not differ significantly from the other samples and the 95% confidence limits of AII and the other samples overlapped.

The estimates for total viability and female fecundity are also largely in agreement with the results of the last chapter (Table

Table 3.12: The relative female fecundity estimates for Cages A and B samples. The 95% confidence limits are given in parentheses.

Cage no.	Female fecundity			G	df	p
	ST/ST	INV/ST	INV/INV			
AI	0.65 (0.30,1.33)	1	1.01 (0.51,1.92)	0.65	2	>0.05
AII	0.63 (0.32,1.18)	1	0.93 (0.45,1.73)	0.76	2	>0.05
AIII	0.84 (0.52,1.55)	1	0.85 (0.41,1.49)	0.14	2	>0.05
Apooled	0.67 (0.43,0.98)	1	0.99 (0.67,1.44)	1.52	2	>0.05
BI	0.55 (0.19,1.01)	1	0.93 (0.37,1.86)	0.84	2	>0.05
BII	1.15 (0.50,2.29)	1	0.79 (0.39,1.70)	0.39	2	>0.05
BIII	0.68 (0.26,1.28)	1	0.81 (0.49,1.22)	0.94	2	>0.05
Bpooled	0.77 (0.52,1.12)	1	0.76 (0.50,1.1)	1.15	2	>0.05
A+B	0.72 (0.54,0.94)	1	0.86 (0.64,1.14)	2.25	2	>0.05

Table 3.13: The relative egg to larval viability estimates for Cages A and B samples. The 95% confidence limits are given in parentheses.

Cage no.	Egg-larval viability			G	df	p
	ST/ST	INV/ST	INV/INV			
AI	2.86 (0.98,5.44)	1	0.77 (0.26,1.54)	10.19	2	<0.01
AII	3.13 (1.86,5.14)	1	0.94 (0.45,1.73)	17.29	2	<0.001
AIII	3.12 (2.1,5.89)	1	0.94 (0.51,1.60)	13.40	2	<0.01
Apooled	2.86 (0.78,3.56)	1	0.69 (0.31,2.06)	39.14	2	<0.001
BI	1.54 (0.88,2.21)	1	0.82 (0.4,1.73)	2.00	2	>0.05
BII	1.28 (0.82,2.47)	1	0.81 (0.33,1.61)	1.10	2	>0.05
BIII	0.85 (0.31,1.2)	1	0.32 (0.16,0.59)	7.09	2	<0.05
Bpooled	1.16 (0.83,1.64)	1	0.65 (0.41,0.97)	4.64	2	>0.05
A+B	1.85 (1.49,2.34)	1	0.67 (0.48,0.89)	32.90	2	<0.001

Table 3.14: The relative zygotic to larval viability estimates (including female fecundity) for Cages A and B samples. The 95% confidence limits are given in parentheses.

Cage no.	Zygotic-larval viability					
	ST/ST	INV/ST	INV/INV	G	df	p
AI	1.89 (0.67,4.2)	1	0.79 (0.18,2.08)	2.48	2	>0.05
AII	1.72 (1.03,2.87)	1	0.36 (0.11,0.94)	4.51	2	>0.05
AIII	2.63 (1.68,4.33)	1	0.79 (0.29,2.07)	4.85	2	>0.05
Apooled	2.0 (1.43,2.71)	1	0.68 (0.42,1.07)	9.91	2	<0.01
BI	0.90 (0.40,2)	1	0.69 (0.23,1.36)	0.39	2	>0.05
BII	1.52 (1.03,2.35)	1	0.62 (0.41,0.94)	1.85	2	>0.05
BIII	0.52 (0.29,0.96)	1	0.29 (0.12,0.63)	5.60	2	>0.05
Bpooled	0.89 (0.64,1.21)	1	0.51 (0.32,0.77)	4.45	2	>0.05
A+B	1.35 (1.07,1.69)	1	0.58 (0.39,0.79)	10.65	2	<0.01

Table 3.15: The relative larval to adult viability estimates for Cages A and B samples. The 95% confidence limits are given in parentheses.

Cage no.	Larval-adult viability			G	df	p
	ST/ST	INV/ST	INV/INV			
AI	0.26 (0.11,0.6)	1	0.36 (0.18,0.79)	12.16	2	<0.01
AII	0.24 (0.1,0.49)	1	1.28 (0.31,2.52)	15.16	2	<0.001
AIII	0.24 (0.14,0.58)	1	0.43 (0.15,0.89)	14.91	2	<0.001
Apooled	0.25 (0.13,0.39)	1	0.56 (0.24,0.75)	39.07	2	<0.001
BI	0.22 (0.11,0.46)	1	0.31 (0.13,0.7)	12.34	2	<0.01
BII	0.23 (0.09,0.62)	1	0.74 (0.36,1.44)	10.66	2	<0.01
BIII	0.87 (0.54,1.20)	1	2.13 (0.9,3.65)	3.28	2	>0.05
Bpooled	0.38 (0.22,0.7)	1	0.85 (0.61,1.39)	14.53	2	<0.001
A+B	0.31 (0.23,0.39)	1	0.70 (0.55,0.92)	49.22	2	<0.001

3.16). Pronounced heterosis was seen in every sample (although non-significantly in Cage A) and st/st individuals always possessed the lowest viability. (In Table 3.16 the values to four decimal places for the st/st and inv/inv karyotypes in the sample pooled over both cages were 0.4178 and 0.4225 respectively.) The samples pooled for Cage B and for both cages both show this effect significantly. It seemed safe to assume that this effect is due to selection between the larval and adult stages.

In contrast to the results of Chapter 2 the male mating success estimates showed no statistically significant advantage to a specific karyotype except in sample AI (Table 3.17). As discussed earlier this was an advantage to inv/st males with inv/st females. This advantage is also reflected, although non-significantly, in the pooled data for Cage A. There did seem to be a mating advantage to homokaryotypic males by the third sample in both cages which failed to be significant. This was also the situation for male mating success in the data pooled for Cage A and across Cages A and B.

To maximise the total viability of their offspring females should mate with males of a certain karyotype. As in Chapter 2, most adult females were heterokaryotypic. To maximise the viability of their progeny they should therefore have mated mainly with males belonging to the homokaryotype category with highest total viability. By this reasoning in every sample in both cages the pattern of mating seemed to be maladaptive except in sample BII and in the sample pooled across both cages (Table 3.18). However, as there were no significant differences in male mating success except in sample AI, where there were no significant differences in total viability, the term 'maladaptive' seems somewhat inappropriate. Sample AI did show significant differences between karyotypes in larval to adult viability. Consequently one could justifiably use the term 'maladaptive' to describe the pattern of mating in AI, if females are expected to mate in order to maximise the fitness of their offspring for this component. Generally though these experiments provided no

Table 3.16: Estimates of relative total viability and female fecundity for Cages A and B samples. The 95% confidence limits are given in parentheses.

Cage no.	Zygote-adult viability					
	ST/ST	INV/ST	INV/INV	G	df	p
AI	0.48 (0.20,1.02)	1	0.33 (0.10,0.84)	3.95	2	>0.05
AII	0.41 (0.21,0.55)	1	0.47 (0.29,0.73)	2.86	2	>0.05
AIII	0.64 (0.37,1.4)	1	0.34 (0.15,0.71)	3.14	2	>0.05
Apooled	0.49 (0.30,0.75)	1	0.39 (0.24,0.64)	0.02	2	>0.05
BI	0.16 (0.05,0.48)	1	0.23 (0.11,0.52)	8.86	2	<0.05
BII	0.35 (0.12,0.68)	1	0.47 (0.22,0.99)	3.38	2	>0.05
BIII	0.45 (0.22,0.93)	1	0.61 (0.28,1.2)	2.58	2	>0.05
Bpooled	0.34 (0.22,0.55)	1	0.45 (0.28,0.72)	11.96	2	<0.01
A + B	0.42 (0.30,0.55)	1	0.42 (0.30,0.57)	19.89	2	<0.001

Table 3.17: The relative male mating success estimates for Cages A and B samples. The 95% confidence limits are given in parentheses.

Cage no.	Male mating success			G	df	p
	ST/ST	INV/ST	INV/INV			
AI	0.1	1	0	9.44	2	<0.01
AII	0.89 (0.27,2.28)	1	0.55 (0.30,3.15)	0.40	2	>0.05
AIII	0.88 (0.38,1.80)	1	2.94 (0.98,6.81)	2.09	2	>0.05
Apooled	0.57 (0.25,1.2)	1	0.73 (0.24,1.73)	1.80	2	>0.05
BI	0.55 (0.02,4.31)	1	0.37 (0.01,2.87)	0.95	2	>0.05
BII	1.70 (0.42,5.20)	1	1.75 (0.40,5.08)	0.93	2	>0.05
BIII	1.64 (0.45,4.90)	1	0.77 (0.21,2.07)	1.06	2	>0.05
Bpooled	1.35 (0.62,2.65)	1	0.93 (0.39,2.02)	0.59	2	>0.05
A+B	1.23 (0.73,1.97)	1	1.56 (0.90,2.55)	2.07	2	>0.05

Table 3.18: 'Adaptiveness' of mating (explanation in text).

Cage sample	Expected mate	Observed mate
AI	st/st	inv/st
AII	inv/inv	inv/st
AIII	st/st	inv/inv
A pooled	st/st	inv/st
BI	inv/inv	inv/st
BII	inv/inv	inv/inv
BIII	inv/inv	st/st
B pooled	inv/inv	st/st
A+B pooled	inv/inv	inv/inv

significant evidence relating to the adaptiveness or otherwise of female mating patterns.

Discussion

The first question which was addressed by this study was whether the phenomena documented in Chapter 2 were seen again. The major finding of Chapter 2 was strong heterosis in larval to adult viability. In addition there were differences in zygotic to larval viability (including female fecundity) between karyotypes. However in this component either st/st or inv/st individuals were favoured in vial samples and in the cages.

In common with Chapter 2, strong heterosis was observed between larval and adult stages which particularly penalised the st/st karyotype. Yet again this effect translated into similar heterosis in total viability. The three-way table analyses on egg and larval data as well as larval and adult data uncovered considerable heterogeneity between cages. As was the case in Chapter 2 however there was no convincing evidence of frequency-dependent viability. It may be that recessive mutations reducing fitness between larval and adult stages accumulated through inbreeding on the inverted sequence and to a greater extent on the standard sequence.

Again, as in Chapter 2 the vial and cage estimates of zygote to larval viability (including female fecundity) were similar which would not have been expected if there was increased larval competition in the cages. Also an advantage to carriers of the standard chromosome arrangement was seen between zygotic and larval stages as in Chapter 2. The selection that occurred between zygotes and larvae in Cages A and B seemed to reflect selection between egg and larval stages. Generally speaking then the findings of these studies on viability have confirmed the results discussed in the last chapter. This was not so for the mating data.

The analysis of mating patterns in Chapter 2 gave two main results. Firstly that mating patterns were non-random in three out of five cages and secondly that st/st males did not always have an advantage over the other two karyotypes in mating.

Although mating was non-random in Cage A, it marginally failed to be so in Cage B. However there did appear to be a similar pattern of change in mating pattern over time in both cages. This consisted of assortative mating between heterokaryotypes in the second generation to occupy the cage (i.e. the first sample), which had changed to an advantage for homokaryotype males by the fourth generation, and had increased by the sixth. (Over the same time matings involving inv/st males and st/st females increased.) The inv/inv males were favoured in this way in Cage A (significantly) but the st/st males were favoured in Cage B (marginally non-significantly). Thus mating appeared to become progressively more disassortative. Such mating patterns are not completely at odds with the results of the previous chapter: assortative mating between heterokaryotypes was seen (although non-significantly) as well as disassortative mating between inv/st females and st/st males.

Although a significant advantage in male mating success was found in one sample from Cage A this was attributable to an advantage to inv/st males with inv/st females. Mating success was not significantly different between karyotypes in any Cage B samples. The increases in matings involving homokaryotype males, seen in the mating patterns of both cages, failed to appear as significant differences in mating success between karyotypes. As a result no reliable conclusions could be drawn about the consequences of any female choice which may have operated to bring about differences in male mating success. In the only sample which had a significant mating advantage (AI) there were no significant differences between karyotypes in total viability.

In conclusion it would seem that the viability heterosis observed in Chapter 2 is stable, at least over roughly six generations. In

addition it would seem that this heterosis maintains the *In(3R)P* polymorphism in the presence of non-random mating patterns which could conceivably lead to the loss of the inversion.

As in the last chapter these findings echo the earlier work of Barnes and Merrell (1985). Having found evidence of non-random mating and heterosis in an inbred population, it was of some interest to test for similar effects in a relatively outbred population polymorphic for the same inversion. If inbreeding was involved in any of the effects seen here we may expect them to be lessened or absent in the outbred populations.

Chapter 4: Inversion polymorphisms in a natural population of Californian *D. melanogaster*

On the basis of their distribution and abundance, inversions in *D. melanogaster* have been divided into four categories (Mettler *et al*, 1977):
(i) Common cosmopolitan inversions (of which there are four: *In(2L)t*, *In(2R)NS*, *In(3L)P* and *In(3R)P*) are said to be found in all natural populations, often at frequencies exceeding those of the standard sequences.
(ii) Rare cosmopolitan inversions (the following six: *In(2R)Cy*, *In(3L)M*, *In(3R)C*, *In(3R)K*, *In(3R)Mo* and *In(3R)M*) are also distributed very widely but fail to reach the ubiquity of the common cosmopolitan inversions. In addition these inversions are rarely found at frequencies as high as the common cosmopolitan inversions.
(iii) Recurrent endemic is the category to which inversions are assigned which are usually infrequent in a population (i.e. frequencies of a few percent) but are found in natural populations some distance apart, or in the same population over several years.
(iv) Rare endemic inversions are the most common type of inversion found in natural populations. Typically these inversions are known only from one population sample and appear at very low frequency.

Data on the frequency of cosmopolitan inversions have been reported from many regions of the world. From the large literature on the (experimental and natural) population dynamics of these inversions there is strong evidence that the frequencies they attain are positively correlated with temperature. Most notably, work done on natural populations in North America, Australasia and Japan have revealed latitudinal clines in the frequency of these inversions (reviewed by Lemeunier *et al*, 1986). Seasonal changes in frequency which mimic the latitudinal clines have also been documented: higher frequencies are found in populations sampled during warmer months (e.g.

Inoue, 1979).

In order to construct a population of flies which had the inversion *In(3R)P* segregating within it and was outbred (relative to the populations investigated in Chapters 2 and 3) it was first necessary to characterise the inversions present in a number of isofemale lines. Then the experiments carried out in Chapter 3 could be repeated using a population derived from as large a number of lines containing *In(3R)P* as possible. Differences in the way the polymorphism was maintained between these relatively inbred and outbred populations could then be sought (see Chapter 5).

Materials and Methods

The 88 *D. melanogaster* isofemale lines examined were derived from females collected during early September 1992 at the Chateau St. Jean winery in Kenwood, California. These isofemale lines were maintained in the same way as the vial stocks in Chapter 2. Twenty third instar larvae were sampled from each line. Larval salivary glands were dissected and mounted as described in Chapter 2. Inversions were located and identified using Bridges' (1935, depicted in Lefevre, 1976) polytene chromosome reference maps in combination with Lindsley and Grell' s (1967) classification of genetic variation. All photomicrographs were taken using the PM-10AD Olympus Photomicrographic System.

Results

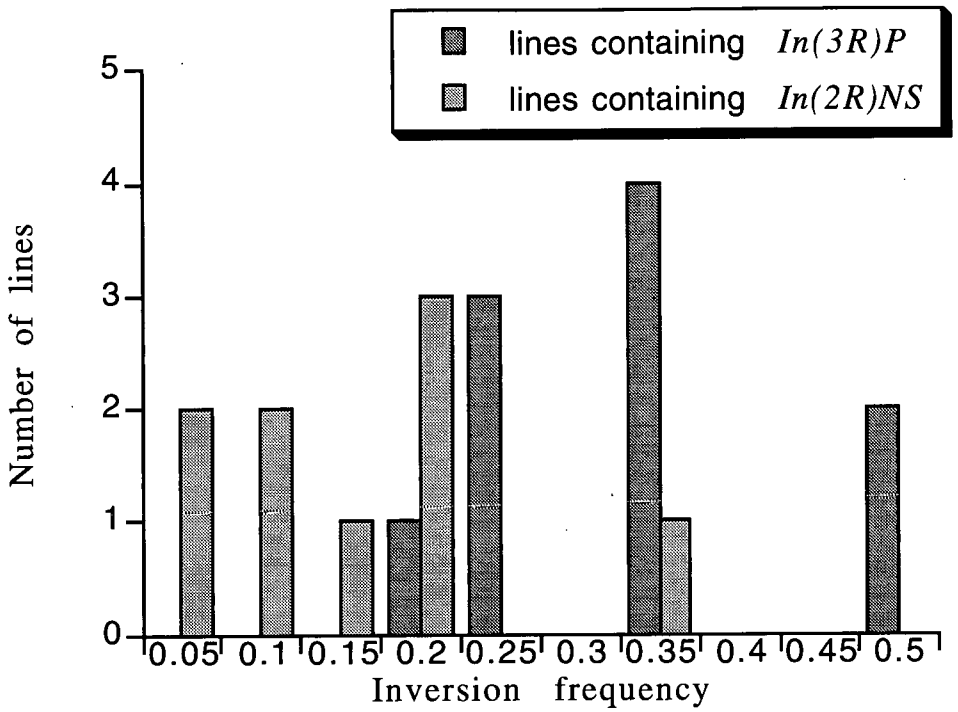
Of the 88 lines examined 22 had an inversion segregating and one line contained two (Table 4). All the other lines (74%) were found to be fixed for the standard (non-inverted) arrangement. Four paracentric inversions were identified, three common cosmopolitan inversions: *In(2L)t*, *In(2R)NS* and *In(3R)P*, and one rare cosmopolitan inversion: *In(3R)Mo*. The photomicrographs of *In(3R)Mo* were unclear (as the chromosomes were distributed

over different field depths). Those made of the others are displayed in Figure 3, Appendix 4. Of these only the common cosmopolitan inversions *In(2R)NS* and *In(3R)P* were found in more than four lines. The distributions of the frequencies attained by these inversions in the lines that they occupied are shown in Plot 1.

Table 4: The inversions identified (frequencies are the number of copies of the inversion in 20 individuals).

Line no.	Inversion Frequency			
	In(2L)t	*In(2R)NS*	*In(3R)Mo*	*In(3R)P*
2	0.20	0.10		
4				0.25
10				0.50
11	0.30			
12	0.25			
13		0.20		
15				0.50
16				0.35
20		0.05		
23				0.35
29				0.25
30	0.05			
33		0.20		
38				0.35
45		0.05		
48		0.35		
49		0.15		
60		0.20		
61				0.25
68		0.10		
70			0.10	
87				0.35
90				0.20

The distribution of inversion frequencies



Plot 1: distributions of the frequencies of *In(2R)NS* and *In(3R)P* .

Discussion

Various questions can be asked of this kind of data on inversion frequencies. For example, the observed karyotypic frequencies in each line can be compared with the expectations at Hardy-Weinberg (H-W) equilibrium. It was originally assumed that such a comparison could detect viability differences between karyotypes. As long as there was no assortative mating, any significant deviations were interpreted as evidence of selection. This approach was taken by several studies of apparent vaibility heterosis in natural populations, for example those by Lewontin and White (1960), Richmond and Powell (1970) and more recently Das and Singh (1990). However as Lewontin (1974) has pointed out, this procedure has no statistical power over realistic selection values (i.e. less than 10% viability differences) for two reasons. The first reason is that the allele frequencies that are used to calculate the H-W proportions are calculated from genotypes which have undergone selection. So, the procedure will test whether $(W_{AA})(W_{aa})=(W_{Aa})^2$ rather than whether the fitnesses of the three genotypes are equal. This includes equal fitnesses between the genotypes but also a variety of multiplicative fitness relations, such as $W_{AA}=1.00$, $W_{Aa}=0.90$ and $W_{aa}=0.81$. There will therefore be little power to discriminate between cases of intermediate dominance under weak selection and equal fitnesses. The second reason the procedure can be misleading is that when it indicates heterozygote excess this does not necessarily mean heterosis, but rather that $(W_{AA})(W_{aa})<(W_{Aa})^2$. So that intermediacy of the heterozygote fitness will present itself as heterosis. (An excess of heterozygotes would be suggestive of viability heterosis only if gene frequencies were not changing.) Nevertheless the type of selection most easily detected by this procedure is viability heterosis, but this must be strong. Viability differences of 10% require a sample size of 4000 to be 90% sure of detection by this method (Lewontin, 1974).

In view of the analysis discussed above there seemed little point

in trying to measure departures from H-W proportions in sample sizes of 20 larvae.

Had there been more data on lines containing more than one inversion one could have tested for non-random associations between inversions, indicating epistatic interactions. Such interactions have been reported in some *D. melanogaster* populations (e.g. Inoue & Watanabe, 1979; Knibb *et al*, 1981), while their absence has been noted in others (Singh & Das, 1990).

Chapter 5: The maintenance of the *In(3R)P* polymorphism in a relatively outbred population.

The history of research into heterosis began with work investigating yield in corn (for example Schull, 1908 and East, 1908). Even at this early stage there was discussion as to the cause behind the observed heterosis. Was this effect attributable to genuinely heterotic loci (overdominance), or to associations with deleterious recessive alleles (dominance)? This question has remained largely unanswered. The early work with corn was at first interpreted as evidence for overdominance, but later longer term experiments implicated (partial or complete) dominance as a more likely explanation (e.g. Gardner, 1963). Evidence collected to support the neutral theory has also indicated that dominance may be responsible for heterosis in other organisms. For example elevations in the number of genes at low rather than intermediate frequencies have been taken to mean that these genes are held at low frequencies by mutation-selection balance (Yamazaki & Maruyama, 1971). Kimura (1983, p271) has presented evidence of high levels of variability in some haploid organisms, which is also evidence against overdominance in these cases.

In *Drosophila* the use of balancer chromosomes by Sved and his colleagues, amongst others, uncovered depressions in the fitness of chromosomal homozygotes of about 80%, relative to heterozygotes (reviewed in Simmons and Crow, 1977)[2] . Sved's work was important because it provided an innovative way to obtain data on the total fitness of chromosomes. The nature of this data made the explanation of heterosis in *Drosophila* all the more pressing. An attempt was made by Wilton *et al* (1989) to resolve this problem using balancer chromosomes. The intention was to derive 'purged'

[2] A description is given of a related experiment undertaken to measure the net fitness of third chromosomes in Appendix 3. This experiment occupied the author prior to the work presented here on inversion polymorphism.

homozygote lines having fitnesses equal to the appropriate heterozygotes by selection against any deleterious genes. Although the results demonstrated no unequivocal increase in homozygote fitness, this did not provide conclusive evidence against heterosis caused by associations with deleterious genes. It was not possible to rule out such associations if dominance was assumed to have been nearly complete.

Several studies have demonstrated the maintenance of inversion polymorphism in caged laboratory populations of *D. melanogaster* (see introduction to Chapter 3). In addition, many studies have reported heterosis associated with inversions (see introduction to Chapter 2). Surprisingly though, there have been few studies that have aimed to characterise the relative importance of dominance and overdominance in heterosis associated with inversions. (As explained in Chapter 2 it is customary to use the terms 'chromosomal' and 'genic' respectively to describe these forms of heterosis.) The experiments of Dobzhansky and his coworkers (eg. Dobzhansky & Levene, 1951; Dobzhansky & Pavlovsky, 1953) have been interpreted as indicating the importance of genic heterosis and coadaptation between arrangements in *D. pseudoobscura* (see Chapter 1). This work was extended (Wasserman, 1968, 1972; Wasserman & Koepfer, 1975) to examine the relative contributions of genic and chromosomal heterosis, as well as the importance of 'supergenic' selection (defined as the disruption of coadapted complexes in homokaryotypes due to recombination) in determining karyotypic viability and egg hatch. This work detected a large genic effect and a small but significant supergenic effect in *D. subobscura*, and a genic effect in *D. pseudoobscura*. On the other hand, work done on the seaweed fly (*Coelopa frigida*) has emphasised the importance of chromosomal heterosis, and asserts that genic heterosis is weak if present at all (Butlin & Day, 1985).

As was mentioned in Chapter 3, more recent work has shown that

the number of isofemale lines used to initiate the cage determines whether the inversion will be maintained or lost. Cages started with one or two isofemale lines (or isofemale lines kept in seperate vials) maintained the inversion, whereas those started with between 40 and 600 lines usually lost it. This has been referred to as the 'cage effect' (Inoue & Watanabe, 1992). This applied to all four common cosmopolitan inversions regardless of differences in food medium quality or temperature. For *In(3R)P* this loss occurred over less than 60 generations (Inoue & Watanabe, 1992).

There are various possible explanations for these observations. Different deleterious recessive alleles may have built up on the standard and inverted sequences causing chromosomal heterosis. This effect would be expected to maintain the polymorphism with a population composed of only one or two isofemale lines. This could continue as long as there were no other standard and inverted chromosomes (carrying yet other deleterious recessives) present, as with a population composed of a larger number of lines. Then many of the homokaryotype individuals produced would not necessarily be homozygous for deleterious recessive genes and the heterosis would lessen. In addition the standard and inverted sequences would be free to recombine with chromosomes which have the same gene arrangement but a different genic content. This would allow selection the opportunity to remove deleterious genes from both sequences, which again should reduce any observed heterosis. Selection against the inversion may be expected as inversions have often been found to contain deleterious genes in natural populations (Lemeunier & Aulard, 1992). The inverted sequence may therefore have more deleterious genes to lose than the standard sequence as well as having less opportunity to recombine (inversion homokaryotypes are invariably rare). An inversion could then be lost from the population by selection against it, aided by drift .

Alternatively, as Inoue and Watanabe (1992) suggest,

recombination between sequences with the same gene arrangement may break up adaptive sequences on the inverted chromosomes. This, they assert, would lead to the loss of the inversion from the population. However, as the authors admit, this fails to explain why certain inversions (*In(2L)t* and *In(2R)NS*) were maintained in a minority of cages.

It was hoped that the experiments presented here, in combination with those in previous chapters, might shed light on the question of the importance of chromosomal heterosis in the maintenance of the *In(3R)P* polymorphism. Additionally the phenomena responsible for the maintenance of inversions in inbred populations and the loss of inversions in relatively outbred populations could be investigated.

Materials and Methods

As in the experiments described in the Chapter 3 the two cages were initiated by an egg sample laid over 24 hours by 150 female flies. These females were randomly selected from the ten isofemale lines polymorphic for the inversion that were characterised in Chapter 4. Again, two replicate cages were allowed to continue for about 6 generations; samples were taken every 21 days (about 2 generations) beginning 21 days after initiation. In total three samples were taken from each cage. Egg samples were not taken during these experiments because of the limited time they were performed over.

Results

Similarity between the cages

The inversion was found at frequencies approximating 0.2 or 0.3 in every cage sample of larvae (CI: 0.31; CII: 0.20; CIII: 0.18; DI: 0.21; DII: 0.18; DIII: 0.17). The decline in the frequency of the inversion

between larval samples was not significant in Cage D but was in Cage C. More precisely sample CI differed significantly from samples CII ($G=8.46$ with 2df, $p<0.05$) and CIII ($G=10.82$ with 2df, $p<0.01$) because it contained more inv/inv and less st/st individuals. Sample CI also differed significantly from sample DI for the same reason, added to the fact that DI contained more inv/st larvae ($G=7.35$ with 2df, $p<0.05$). (However there were no other differences between sequentially equivalent larval samples from the cages.) A distinct trend was also seen in the comparisons made between larval frequencies and H-W expectations. In every sample (and the samples pooled across cages) there were significant differences (see Table 5.2) caused by higher numbers of inv/inv and inv/st larvae than expected, in combination with lower numbers of st/st larvae than expected. In both cages, in every sample, there were no significant differences between adult male and female counts. In Cage C there were also no significant differences between successive adult samples but this was not the case with Cage D. In sample DIII there were significantly more inv/st and less homokaryotype adults than in DI ($G=11.03$ with 2df, $p<0.01$) and DII ($G=8.89$ with 2df, $p<0.05$). There were no significant differences between sequentially equivalent samples from Cages C and D (although the difference between samples CIII and DIII was only marginally non-significant at the $p<0.05$ level ($G=5.88$ with 2df), again because of more inv/st and less homokaryotype adults in DIII). There were also no significant differences between the three mating frequency samples from Cage C. In Cage D there was only one significant difference: between samples DII and DIII ($G=18.39$ with 8df, $p<0.05$). This was due to more matings between st/st males and inv/st females in DIII. There were also more matings of this type in sample CII than in DII ($G=16.16$ with 2df, $p<0.05$), and this constituted the only significant difference in mating pattern between sequentially equivalent samples from the cages. The mating patterns pooled across cages failed to differ significantly.

Raw data

Table 5.1: Larval and adult counts from Cages C and D.

Cage Sample	Larvae			Adults		
	ST/ST	INV/ST	INV/INV	ST/ST	INV/ST	INV/INV
CI	26	56	17	14	35	10
CII	40	55	6	16	17	6
CIII	45	51	6	19	23	5
pooled	111	162	29	49	75	21
DI	43	48	9	12	21	11
DII	45	48	6	17	19	7
DIII	48	36	6	10	29	1
pooled	136	132	21	39	69	19

Table 5.2: Summary of results of test for departure from Hardy-Weinberg equilibrium.

Cage sample	G	df	p
CI	6.28	1	<0.05
CII	12.85	1	<0.001
CIII	10.52	1	<0.01
C pooled	31.28	1	<0.001
DI	7.92	1	<0.01
DII	9.36	1	<0.01
DIII	5.35	1	>0.05
D pooled	22.11	1	<0.001

Table 5.3: Matings from Cages C and D. Corrected matings shown in parentheses (see Appendix 1).

Cage Sample	Mating	♀ ST/ST	♀ INV/ST	♀ INV/INV
CI	♂ ST/ST	0 (0)	7 (6.1)	2 (1.96)
CII		0 (0)	6 (5.97)	0 (0)
CIII		3 (2.93)	4 (3.19)	0 (0)
DI		1 (0.96)	4 (3.17)	2 (1.97)
DII		2 (1.89)	1 (0.46)	0 (0)
DIII		0 (0)	9 (8.06)	0 (0)
CI	♂ INV/ST	5 (5)	10 (12.2)	5 (5.04)
CII		6 (6.06)	1 (1.03)	2 (2)
CIII		9 (9.07)	5 (6.26)	2 (2.01)
DI		5 (5.04)	4 (4.83)	4 (4.03)
DII		8 (8.11)	4 (4.75)	2 (2)
DIII		5 (5)	5 (5.94)	0 (0)
CI	♂ INV/INV	0 (0)	3 (1.70)	0 (0)
CII		1 (0.94)	0 (0)	0 (0)
CIII		0 (0)	1 (0.55)	1 (0.99)
DI		0 (0)	0 (0)	0 (0)
DII		0 (0)	3 (2.79)	0 (0)
DIII		0 (0)	0 (0)	0 (0)

Table 5.4: Corrected matings inferred for adult females from Cage C (the numbers expected if mating occurred at random are given in parentheses).

	♀ ST/ST	♀ INV/ST	♀ INV/INV
♂ ST/ST	2.92 (8.45)	15.46 (13.03)	1.97 (4.23)
♂ INV/ST	20.16 (12.85)	19.35 (19.80)	9.07 (6.42)
♂ INV/INV	0.92 (2.70)	2.19 (4.17)	0.96 (1.35)

Table 5.5: Corrected matings inferred for adult females from Cage D (the numbers expected if mating occurred at random are given in parentheses).

	♀ ST/ST	♀ INV/ST	♀ INV/INV
♂ ST/ST	2.78 (5.64)	12.14 (8.05)	1.97 (2.15)
♂ INV/ST	18.22 (11.91)	15.56 (17.02)	6.03 (4.54)
♂ INV/INV	0 (3.45)	2.3 (4.93)	0 (1.31)

Table 5.6: Corrected matings inferred for adult females from Cages C and D (the numbers expected if mating occurred at random are given in parentheses).

	♀ ST/ST	♀ INV/ST	♀ INV/INV
♂ ST/ST	5.81 (14.02)	27.58 (20.88)	3.94 (6.23)
♂ INV/ST	38.31 (24.78)	35.23 (36.90)	15.12 (11.01)
♂ INV/INV	0.88 (6.20)	4.19 (9.23)	0.94 (2.75)

In summary it appeared that over six generations both cages underwent a increase in the frequency of st/st larvae (Cage C going from 26% to 44% and D from 43% to 53%) with a corresponding drop in the frequencies of the other two karyotypes. This trend was however only significant in Cage C. Despite this decline in st/st individuals there were still fewer representatives of this karyotype than expected under H-W equilibrium in every sample. A similar trend was observed in adult karyotypic frequencies in both cages with the exception of sample DIII, but failed to be significant. The (rather limited) heterogeneity in mating patterns did not follow a consistent trend. It seemed acceptable to describe the cages as replicates. However, the differences between samples, particularly in larval frequencies, emphasises the need for caution in interpreting the data pooled across cages.

Heterosis and non-random mating

Significant heterosis was observed only once: in sample DIII between larval and adult stages (see Table 5.13). This contrasted with the inv/inv advantage which was the only significant result in other samples for larval to adult viability. Differences between karyotypes in this fitness component will be discussed further below.

Mating patterns which were non-random were observed in both cages (Table 5.7). (The definition of 'random' mating was the same as in the preceeding two chapters.) In sample CII mating was non-random due to disassortative mating between inv/st and st/st individuals. This phenomenon was also responsible for the departure from random mating in the Cage C pooled sample, as well as an increase in matings involving inv/st males and inv/inv females. Similar patterns were observed in Cage D. Sample DII exhibited non-random mating as a result of increased matings between inv/st males and both homokaryotype females. In sample

Table 5.7: Summary of results of test for non-random mating.

Cage sample	G	df	p
CI	7.93	6	>0.05
CII	19.83	6	<0.01
CIII	6.65	6	>0.05
C pooled	13.58	6	<0.05
DI	10.18	6	>0.05
DII	13.72	6	<0.05
DIII	11.26	6	>0.05
D pooled	18.12	6	<0.01
C and D pooled	28.40	6	<0.001

Three-Way Table Analysis

Table 5.8: Summary of the analysis examining two and three way interactions in the mating data (explanation in text).

Interaction	G	df	deviates
♀ karyotype by ♂ karyotype by cage	2.11	4	<
♀ karyotype by cage	2.51	6	<
♀ karyotype by ♂ karyotype	15.78	8	>
♂ karyotype by cage	2.34	6	<

DIII (which was significantly non-random only at the $p<0.10$ level) disassortative mating between inv/st and st/st individuals was observed. The pooled Cage D mating sample was consequently non-random because of disassortative mating between the inv/st and st/st karyotypes and an increase in matings involving inv/st males and inv/inv females. Thus the non-random pattern of mating in samples pooled across each cage appeared to be the same. These trends in mating are also responsible for the significant departure from random mating seen in the matings pooled across both cages.

Three-way table analyses

A three-way analysis of the mating data from Cages C and D revealed no significant interactions other than between male and female karyotype (Table 5.8). Two Freeman-Tukey deviates exceeded the critical value for this interaction (see the explanation given in previous chapters) indicating that the non-random mating present was not identical in the two cages.

A three-way analysis of zygotic and larval frequencies gave no significant associations, supporting a lack of selection in either cage between these stages. In contrast the same test applied to larval and adult frequencies identified two significant associations (Table 5.9). Cage and karyotypic frequency were found to be associated and this was a result of the differences between cages and between samples from the same cage. These differences were of the kind already discussed. For example the frequency of st/st larvae in the first Cage D sample was almost twice that of the first Cage C sample. In addition karyotypic frequencies in larvae and adults changed over the three samples from each cage. The more significant interaction term for this data was between data type and karyotypic frequencies. This was mainly attributable to inv/inv individuals increasing, and st/st individuals decreasing, their representation between larval and adult stages. This provides further evidence for

Table 5.9: Summary of the analysis examining two and three way interactions in the larval and adult frequencies data (explanation in text).

Interaction	G	df	deviates
cage by count by karyo freqs	17.16	10	>
cage by count	19.38	15	>
cage by karyo freqs	42.19	20	>
count by karyo freqs	42.42	12	>

Table 5.10: Summary of the analysis examining two and three way interactions in the zygotic and adult frequencies data (explanation in text).

Interaction	G	df	deviates
cage by count by karyo freqs	4.77	10	<
cage by count	5.65	15	<
cage by karyo freqs	23.52	20	>
count by karyo freqs	90.20	12	<

a selective advantage to the inv/inv karyotype between these stages. Zygotic and adult frequencies were also tested in the same way (Table 5.10). Again the only significant association was between data type and karyotypic frequencies. This was attributable to the observed trend of weak heterosis in zygotic to larval viability (including female fecundity).

Fitness estimates

Viability estimates were broken down into the relative karyotypic viabilities over certain stages of the life cycle as in the preceeding two chapters. Also in common with previous chapters there were two kinds of estimates for zygotic to larval viability (including female fecundity), one for samples in vials (made to allow the correction of mating data as in Appendix 1) and one for cages. There were no significant differences between karyotypes for either estimate of this fitness component, although a non-significant disadvantage to inv/inv individuals appeared in every cage estimate (Table 5.12) and most vial estimates (Table 5.11). This advantage was marginally significant at the $p<0.1$ level in the cage estimate data pooled across both cages (Table 5.12). Inspection of the 95% confidence limits for cage and vial samples revealed no evidence (i.e. lack of overlap) for increased larval competition in the cages.

The estimates of larval to adult viability also failed to reveal any significant differences between karyotypes in Cage C (Table 5.13). A non-significant advantage to the inv/inv karyotype did however appear to be present. This advantage was also seen significantly in sample DI and in the pooled sample for Cage D. The significant exception to this phenomenon was sample DIII where heterosis was observed for this fitness component. This heterosis seemed particularly disadvantageous for inv/inv individuals. Needless to say, this effect was not seen in the pooled data for Cage D, where a

Table 5.11: The relative zygotic to larval viability (including female fecundity) estimates for vials at approximately optimum density. All measures of significance are derived from differences in likelihood (see Appendix 1). The 95% confidence limits are given in parentheses.

Cage no.	Zygotic-larval viability					
	ST/ST	INV/ST	INV/INV	ΔL	df	p
CI	0.96 (0.63,1.48)	1	1.14 (0.71,1.87)	0.13	2	>0.05
CII	1.01 (0.64,1.64)	1	0.80 (0.41,2.35)	0.13	2	>0.05
CIII	0.90 (0.57,1.35)	1	0.34 (0.15,1.59)	0.74	2	>0.05
Cpooled	0.93 (0.74,1.21)	1	0.99 (0.68,1.46)	0.23	2	>0.05
DI	1.24 (0.74,2.13)	1	0.88 (0.47,1.75)	0.63	2	>0.05
DII	1.37 (0.87,2.14)	1	1.03 (0.62,1.83)	1.02	2	>0.05
DIII	1.21 (0.84,1.86)	1	0.97 (0.45,2.55)	0.53	2	>0.05
Dpooled	1.24 (0.95,1.65)	1	1.00 (0.64,1.56)	1.77	2	>0.05
C+D	1.06 (0.88,1.29)	1	0.99 (0.74,1.33)	0.31	2	>0.05

Table 5.12: The relative zygotic to larval viability (including female fecundity) estimates for Cages C and D samples. The 95% confidence limits are given in parentheses.

Cage no.	Zygotic-larval viability			G	df	p
	ST/ST	INV/ST	INV/INV			
CI	0.93 (0.52,1.62)	1	0.80 (0.33,1.52)	0.18	2	>0.05
CII	0.99 (0.60,1.65)	1	0.74 (0.34,1.79)	0.08	2	>0.05
CIII	0.88 (0.41,1.08)	1	0.31 (0.11,0.69)	2.47	2	>0.05
Cpooled	0.96 (0.71,1.29)	1	0.56 (0.33,0.90)	2.07	2	>0.05
DI	1.52 (0.89,2.69)	1	0.61 (0.24,1.37)	1.42	2	>0.05
DII	1.14 (0.70,1.9)	1	0.32 (0.09,0.79)	2.64	2	>0.05
DIII	1.59 (0.82,2.75)	1	1.06 (0.45,2.15)	0.80	2	>0.05
Dpooled	1.35 (0.98,1.84)	1	0.58 (0.26,0.99)	3.25	2	>0.05
C+D	0.68 (0.54,0.88)	1	0.57 (0.35,0.81)	5.06	2	>0.05

Table 5.13: The relative larval to adult viability estimates for Cages C and D samples. The 95% confidence limits are given in parentheses.

Cage no.	Larval-adult viability			G	df	p
	ST/ST	INV/ST	INV/INV			
CI	0.86 (0.36,1.81)	1	0.94 (0.37,2.14)	0.14	2	>0.05
CII	1.29 (0.60,1.65)	1	3.23 (0.75,8.16)	3.30	2	>0.05
CIII	0.94 (0.39,2.68)	1	1.85 (0.76,3.96)	1.04	2	>0.05
Cpooled	0.95 (0.61,1.49)	1	1.56 (0.81,2.65)	2.31	2	>0.05
DI	0.64 (0.04,2.08)	1	2.78 (0.62,6.50)	7.23	2	<0.05
DII	0.94 (0.25,4.73)	1	2.94 (0.93,7.29)	3.47	2	>0.05
DIII	0.26 (0.09,0.56)	1	0.21 (0.03,0.45)	12.06	2	<0.10
Dpooled	0.55 (0.32,0.89)	1	1.72 (0.86,3.18)	12.32	2	<0.10
C+D	1.23 (0.87,1.70)	1	1.64 (1.04,2.49)	4.76	2	>0.05

significant advantage for inv/inv individuals was again seen. This advantage was also present in the data pooled across both cages but was significant only at the $p<0.10$ level.

No significant differences in total viability and female fecundity were detected (Table 5.14) but there did appear to be a non-significant trend of weak heterosis in the data. This is more clearly seen in the samples pooled across cages. Male mating success did not differ significantly between karyotypes in Cage C or in any Cage D samples other than DI and the sample pooled over Cage D (Table 5.15). The value of zero for inv/inv male mating success in sample DI meant that the similar pattern shown in the pooled Cage D sample was likely to be more reliable. This pattern, of similar mating success for inv/st and st/st and a disadvantage to inv/inv, also occurred significantly in the data pooled across both cages. A non-significant trend, of increased homokaryotype male mating success in the third sample, appeared in both cages. This trend favoured inv/inv males in Cage C and st/st males in Cage D.

The lack of significant differences in total viability between karyotypes made it impossible to come to any reliable conclusions about the 'adaptiveness' (see previous chapters for definition) of the observed mating patterns (Table 5.16). Alternatively one may assume that females should mate to increase the larval to adult viability component of their offspring. Then in the two samples with significant differences between karyotypes in both larval to adult viability and male mating success (DI and pooled over Cage D) mating was maladaptive.

Comparison with Chapter 3

Various G-tests were carried out in order to obtain statistically evidence of differences in viability and mating patterns between the relatively inbred populations examined in Chapter 3 and those

Table 5.14: Estimates of relative total viability and female fecundity for Cages C and D samples. The 95% confidence limits are given in parentheses.

Cage no.	Zygotic-adult viability					
	ST/ST	INV/ST	INV/INV	G	df	p
CI	0.80 (0.34,1.0)	1	0.75 (0.29,1.68)	0.34	2	>0.05
CII	1.26 (0.42,3.58)	1	2.38 (0.56,7.20)	0.77	2	>0.05
CIII	0.83 (0.35,1.15)	1	0.57 (0.19,1.30)	0.54	2	>0.05
Cpooled	0.92 (0.59,1.43)	1	0.88 (0.45,1.56)	0.12	2	>0.05
DI	1.09 (0.32,2.29)	1	0.92 (0.65,3.09)	0.64	2	>0.05
DII	1.09 (0.41,2.47)	1	0.92 (0.24,2.55)	0.05	2	>0.05
DIII	0.41 (0.20,0.98)	1	0.22 (0.04,0.59)	3.03	2	>0.05
Dpooled	0.74 (0.43,1.20)	1	0.99 (0.50,1.82)	0.78	2	>0.05
C+D	0.83 (0.60,1.17)	1	0.94 (0.61,1.41)	0.62	2	>0.05

Table 5.15: The relative male mating success estimates for Cages C and D samples. The 95% confidence limits are given in parentheses.

Cage no.	Male mating success			G	df	p
	ST/ST	INV/ST	INV/INV			
CI	0.60 (0.21,1.56)	1	0.45 (0.02,1.96)	1.27	2	>0.05
CII	0.74 (0.20,2.60)	1	0.28 (0.02,2.08)	1.15	2	>0.05
CIII	0.66 (0.22,2.59)	1	1.54 (0.62,3.55)	0.66	2	>0.05
Cpooled	0.65 (0.33,1.19)	1	0.41 (0.09,1.19)	2.89	2	>0.05
DI	0.93	1	0	6.28	2	<0.05
DII	0.24 (0.01,1.01	1	0.38 (0.24,1.54)	3.57	2	>0.05
DIII	2.17	1	0	2.51	2	>0.05
Dpooled	0.89 (0.41,1.79)	1	0.18 (0.02,0.73)	6.13	2	<0.05
C+D	0.76 (0.47,1.21)	1	0.27 (0.08,0.67)	8.00	2	<0.05

Table 5.16: 'Adaptiveness' of mating (explanation in text).

Cage sample	Expected mate	Observed mate
CI	inv/st	inv/st
CII	st/st	inv/st
CIII	inv/st	inv/inv
C pooled	inv/st	inv/st
DI	inv/inv	inv/st
DII	st/st	inv/st
DIII	inv/st	st/st
D pooled	inv/st	inv/st
C+D pooled	inv/st	inv/st

investigated here. The vitual absence of larval to adult viability heterosis discussed above was not reflected in a G-test between the fitness indices of the data pooled for Cages A, B, C and D. No significant differences were found between the four cages, nor between the data pooled for Cages A and B and that pooled for C and D. Nevertheless, inspection of the fitnesses (and their confidence limits) calculated in Chapter 3 (Table 3.15) and in this chapter (Table 5.13) does suggest a non-significant trend of increased heterosis in the inbred populations of Chapter 3. Heterosis between the zygotic and adult stages (including female fecundity) also failed to be significantly different between the four cages, although the data pooled across Cages C and D, and Cages A and B, did reveal a non-significant decrease in the severity of heterosis in the outbred populations (Cages C and D).

The mating patterns of the four cages failed to differ significantly except between Cages B and D ($G=16.28$ with 8 df, $p<0.05$). This was because of an increase in Cage D of matings between inv/st males and st/st females and a decrease in those between heterokaryotypes, relative to Cage B. A similar significant difference was seen between the data pooled for Cages A and B, and Cages C and D ($G=21.08$ with 2 df, $p<0.05$). Again there were more matings between inv/st males and st/st females as well as less between heterokaryotypes in the outbred populations. In addition the matings with inv/st females increased for st/st males and decreased for inv/inv males in the outbred populations.

Discussion

There were two main questions to be answered by the work presented in this chapter. First, whether heterosis for viability and non-random mating were associated with *In(3R)P* in a population which was relatively outbred, compared to the populations used in Chapters 2 and 3. Secondly, whether either or both of these

agencies were important in predicting the dynamics of the polymorphism.

Zygotic to larval viability (including female fecundity) indicated a rather mild (and non-significant) disadvantage to inv/inv individuals in every cage estimate and most vial estimates. This was reminiscent of the results for Chapters 2 and 3 where the same effect was seen, though more strongly.

The main finding of these experiments was the virtual absence of heterosis in larval to adult viability. Although heterosis was found to be present in one sample it was not particularly disadvantageous to the st/st karyotype as in Chapters 2 and 3. In the experiments presented here larval to adult viability generally showed an advantage to inv/inv individuals. Correspondingly there were no significant differences in total viability between karyotypes, though there did appear to be a trend of weak heterosis. Again this contrasts with the results of Chapters 2 and 3 where strong heterosis, similar to that between larval and adult stages, was present. However, comparisons with the results of Chapter 3 yielded no significant differences between the severity of heterosis in inbred and outbred populations.

Mating in Cages C and D was non-random due to disassortative mating between the inv/st and st/st karyotypes and an increase in matings involving inv/st males and inv/inv females. This pattern of mating was seen in Chapter 3 (and to a limited extent in Chapter 2) but was accompanied by the observation of assortative mating between heterokaryotypes in the earliest samples. Assortative mating was not seen in Cages C and D. Mating patterns were significantly more disassortative than those observed in the inbred populations of Chapter 3.

In Chapter 3 there were generally no differences in male mating

success. However one sample did show an advantage for inv/st males. Advantages for these males were also the only significant differences in male mating success in Cages C and D. In addition to this both of the cages from Chapter 3 and Cages C and D showed the same non-significant trend in male mating success. A change in the karyotype favoured by differences in male mating success, from inv/st males to homokaryotype males. Interestingly this change favoured st/st males in one replicate and inv/inv males in the other in both this chapter and Chapter 3.

It appears that the consistent deficit of st/st larvae and excess of the other two karyotypes in Cages C and D, relative to H-W expectations, is explicable on the basis of the mating patterns and viability differences that were observed. Viability differences favouring the inv/inv karyotype between larvae and adults would seem to have been the likely cause of this pattern in the larvae. However, the mating advantages to homokaryotypic males which developed in either cage (Table 5.15) also appear to have contributed to the magnitude of such effects in the larvae. In Cage C where an advantage to inv/inv males seemed to develop, the excess of inv/inv and inv/st larvae, at the expense of st/st larvae, increased in magnitude (see Table 5.2). In contrast, in Cage D where a mating advantage to st/st males arose, the deficit in st/st larvae reduced, and was not significant by sample DIII. Nevertheless the inversion frequency did appear to be decreasing between larval samples in both cages. Thus, contrary to Chapters 2 and 3, it would seem that any heterosis associated with *In(3R)P* was sufficiently weak to allow differences between karyotypes in male mating success to affect the frequency of the inversion.

Combining the results presented here, for cages C and D and those from Chapter 3, for Cages A and B, seemed to lead to the following conclusions:

(i) Inbred populations showed strong heterosis associated with

In(3R)P which is not present in relatively outbred populations. This constitutes indirect evidence for chromosomal heterosis acting in the inbred populations. However, the difference in relative karyotypic viabilities failed to be statistically significant.
(ii) Inbred and outbred populations showed similar patterns of mating, particularly the tendency of the inv/st and st/st karyotypes to mate with one another. This kind of disassortative mating was significantly more frequent in outbred populations. This pattern should lead to a decrease in the frequency of the inversion in the absence of any opposing selection favouring the inversion. This decrease was seen in the outbred populations but was overpowered by viability heterosis in the inbred populations.

As was mentioned in Chapter 2, these two effects provide a possible explanation for the Inoue & Watanabe (1992) 'cage effect'. In this case sexual selection may have acted against the inversion in cages started with many isofemale lines. This may be expected if the most common females (inv/st) showed a preference to mate with st/st males. This type of non-random mating appears in both inbred and outbred populations. It therefore becomes unnecessary to postulate the break up of adaptive sequences to explain the removal of the inversion from the relatively outbred populations.

Chapter 6: Simulation of inversion polymorphism as a result of chromosomal heterosis

The conditions under which a decrease in recombination and an increase in linkage disequilibrium at two loci would be favoured were first considered by Fisher (1930, pp102-104). In later years his work formed the basis for a discussion about the causes of inversion polymorphism. This discussion was initiated by Sturtevant and Mather (1938) who first suggested that heterosis might develop from the presence of different disadvantageous genes on the inverted and uninverted complementary sequences. They explained that it was not possible to formulate a detailed algebraic treatment of this phenomenon because there can be no stability in the exact relations of the sequences with each other. The gene content of either sequence always fluctuates over time, but they must be on average the same as each other (or any other sequence in the genome) over a great length of time. At any given moment however the genes carried on the two sequences are different, as each will carry mutations that are lacking in the other.

Mathematical modelling of the fate of inversions in populations was approached more rigorously thirty years later. Information from studies of laboratory and natural populations on the occurrence and dynamics of inversions had built up in the intervening period. In addition to this, the development of computers meant more complex models could be analysed. It was suggested that the evolutionary history of an inversion consists of three stages (Nei *et al*, 1967). The initial survival of an inversion in a population (the first stage) is a stochastic process. This is because the inversion most probably appears in only one individual and so the survival of the inversion's few descendants is subject to stochastic errors. The second stage is the inversion's rise in frequency which is also stochastic in small populations. Once the frequency of the inversion becomes high enough for the loss of all its copies from the population by chance to be unlikely, this stage may be treated deterministically. The

last stage consists of the inversion either becoming established as a balanced polymorphism due to some form of selection; or results in fixation of the inversion due to directional selection or genetic drift.

Additive models

Many of the first models analysed the simplest additive case, neglecting any epistatic interactions between genes. For example, examining the ultimate probability of survival of an inversion Ohta and Kojima (1968) concluded that this would be zero, unless it possessed a constant selective superiority at all times. Cook and Nasser (1972) later extended this work to show that this advantage need not be constant for the inversion's survival, and may in fact decline as long as the decline was sufficiently slow.

Other work centred on how an inversion becomes abundant in a population. Nei *et al* (1967) investigated the increase in frequency of an inversion carrying few deleterious alleles relative to the average number per chromosome. They found that new deleterious mutations occurring on the inversion gradually reduced the selective advantage it enjoyed. The inversion's increase in frequency stopped when the inversion became selectively neutral with respect to the average non-inverted sequence. Another study looked at the likelihood of establishing new inversions (that included 11 loci) with varying allelic contents in small populations of 12 or 24 individuals (Kojima, 1967). This author begins with the assumption that the fitness of inverted chromosomes relative to that of non-inverted chromosomes varies over generations. This variation is a function of the random sampling and selective changes in gene frequencies, as well as the recombination that non-inverted chromosomes are subject to. The establishment of new inversions was therefore treated as a stochastic process, heterogeneous in relation to the fitness parameter. For this reason a Monte Carlo simulation was deemed appropriate. The most important factors that determined whether an inversion

became extinct, fixed or polymorphic were gene action (dominance, absence of dominance and occasionally overdominance were considered), the allelic content of the inversion and the initial frequencies of advantageous alleles in the population. With dominance chromosomal heterosis was seen to develop between inversions carrying a few disadvantageous alleles and non-inverted sequences carrying different disadvantageous alleles at loci complementary to those on the inversions. With the addition of an overdominant locus the likelihood of obtaining a balanced polymorphism was increased. The study was limited by only considering small populations, omitting mutation and by adopting a regime of truncating selection on the phenotypic value (deduced by adding the genotypic value to an environmental 'noise' component).

In another additive model a regime of normalising selection was imposed where an intermediate optimum phenotype is favoured (Fraser *et al*, 1966; Fraser & Burnell, 1967). These studies also found that balanced polymorphism evolved as a result of chromosomal heterosis. Alternatively it could evolve by the inverted and non-inverted sequences combining to give optimal phenotypes through additive effects alone. With 6 loci polymorphism was only attained if the inversion was introduced into a small population at a frequency above 0.12, which is probably biologically unrealisable. Otherwise the inversion was lost from the population. As with Kojima's (1967) study overdominance was a sufficient but not a necessary condition for polymorphism (Fraser *et al*, 1966). When linkage was made looser or the number of loci was increased to 30, polymorphism became more likely (Fraser & Burnell, 1967).

Two Locus Models with Epistasis

Many models investigating the population genetics of inversions have been analysed that incorporate epistatic effects between loci. Research on this question also took Fisher (1930) as its starting point. The simplest approach is to examine the

conditions necessary for the establishment of an allele at a locus which modifies recombination between two other loci under selection. The recombination modifier locus is assumed to be devoid of any effects on fitness. Nei (1967) was first to use this approach and found that in general recombination would tend to be reduced as long as the selected loci were in linkage disequilibrium. Later work looked specifically at the initial increase in frequency of a mutant allele at a modifier locus when the selected loci are in linkage disequilibrium. The allele was found to undergo an initial rise in frequency regardless of the type of selection operating at the selected loci, and of the linkage of the modifier to the other loci (Feldman, 1972; Feldman *et al*, 1980).

This research was accompanied by the idea that recombination reduction is expected, for sufficiently tight linkage between the selected genes, as this will increase the mean fitness of the population (Lewontin, 1974). This remains true as long as there are constant genotypic fitnesses and random mating. Multilocus populations in constant environments should therefore tend towards a total absence of recombination. Evidence from two-locus modelling supports this, but the opposite also seems true (Charlesworth, 1976). That is, high equilibrium levels of recombination were favoured for intermediate values of environmental periodicity. This was also the case for close linkage between the modifier locus and the selected loci and for high levels of environmental variation. Karlin and McGregor (1974) confirmed that for any viability selection system and sufficiently small recombination values, there was an initial increase in a recombination-reducing allele. Feldman *et al* (1980) looked at the situation where the selected loci are held in a balance between selection against deleterious alleles and mutation towards them. They found that if the initial disequilibrium was negative, then increased recombination was favoured provided the modifier and selected loci were sufficiently tightly linked. As this latter linkage decreased the

result reversed and reduced recombination between the selected genes evolved.

Multilocus Models and Exchange Between Gene Arrangements

Haldane (1957) conceived the first multilocus model to investigate how epistasis affected the equilibria attained by an inversion. He found that cumulative heterosis (where heterozygosity at a locus increased fitness more when other loci were heterozygous) was a necessary condition both for the establishment and maintenance of stable polymorphism. Other workers analysed more general models of multilocus systems. For example Deakin (1972) examined various equilibria but failed to find any threshold frequency that was necessary for the inversion to become established (see Fraser *et al*, 1966 and Fraser & Burnell, 1967 above). An important (two, three and five locus) study by Charlesworth and Charlesworth (1973) looked at the dynamics of an inversion introduced into a large population. They discovered that the intensity of selection on the inversion was proportional to the difference between its marginal fitness (that is, the fitness of identical gametes regardless of gene arrangement) and the mean fitness of the population at equilibrium. The inversion rose in frequency as long as certain conditions are met. The population must already be in linkage disequilibrium and the genic content of the inversion had to be that of the gametic type present in excess. When the inversion appeared with the genic content of the gamete found in deficiency it was lost from the population. Thus, in contrast to earlier studies (for example Kojima, 1967 and Fraser & Burnell, 1967), epistasis was found to be necessary but not sufficient for an inversion to become established in a population. A threshold frequency was found to be necessary for the inversion to increase in a population in linkage equilibrium. At this frequency the inversion created linkage disequilibrium simply through its presence. Charlesworth and Charlesworth (1973) also found that the probability of survival of an inversion was approximately one half of the square root of the recombinational

load (the disadvantage which results from breaking up favourable combinations of genes) experienced by the population.

Certain studies have made attempts to examine the equilibria reached by inversions introduced into a two-locus system, when there is exchange between the inverted and non-inverted sequences (Deakin & Teague, 1974; Charlesworth, 1974; Teague & Deakin, 1976). Deakin and Teague (1974) modelled the cases where only one of the genes considered was within the inversion and where the inversion was located between the two loci. It proved difficult to analyse these rather complex models and derive conditions for stable equilibria. They concluded that a generalised two-locus model would also be an accurate description of a multilocus model (Teague & Deakin, 1976).

Charlesworth (1974) began by examining the three types of equilibria possible in a two-locus system without recombination. He showed that these equilibria were dependent upon the fitness effects associated with the loci concerned. One of these equilibria involved the cumulative heterosis effect mentioned above, where the two arrangements were genetically homogeneous (i.e. monomorphic) but differed at both loci. With the other equilibria the non-inverted sequence remained heterogeneous (i.e. polymorphic) for one or both loci. Charlesworth observed that with certain fitness effects there were two alternative equilibria, so that the frequency reached by an inversion was contingent upon the population's history. When some exchange was allowed between the two arrangements the equilibria reached were very close to those attained when there was none. Within each sequence linkage equilibrium developed between the two selected loci, although they were both in linkage disequilibrium with the inversion. Exchange between gene arrangements also allowed the population to remain in a 'quasi-equilibrium' for many thousands of generations. Interestingly in this state the inversion tended not to return to its original frequency after a perturbation. In addition it remains relatively genetically homogenous, particularly if rare.

In summary, the modelling of the origin and maintenance of inversion polymorphism has centred upon two possible mechanisms: the chance accumulation of different genes on different arrangements (chromosomal heterosis), and epistatic interactions between genes. Certain authors have emphasised the importance of chromosomal heterosis (Nei *et al*, 1967; Kojima, 1967; Fraser *et al*, 1966; Fraser and Burnell, 1967; Cook and Nasser, 1972), but this mechanism has yet to be modelled with realistic mutation rates, numbers of loci or population sizes. Other work has introduced epistatic interactions between genes to mimic the inhibitory effect of an inversion on recombination between genes within its breakpoints (Nei, 1967; Feldman, 1972; Feldman *et al*, 1980; Charlesworth and Charlesworth, 1973; Karlin and McGregor, 1974; Charlesworth, 1976). This research has given many important insights into how an inversion may become established in a population. However the question of whether chromosomal heterosis as a result of recombination inhibition and realistic rates of mutation can, by itself, maintain inversion polymorphism has not been addressed directly.

The present model

The program simulates a chromosome carrying n genes (numbered 0 to n-1) in a population of N individuals. Locus 0 arbitrarily marks the inversion arrangement, in that heterozygotes at locus 0 do not recombine. Within an inversion, loci are spaced at map distance r: the total map length between the (n-1) 'ordinary' genes is r(n-2). (The program analysed in this study was written by Professor Nick Barton.)

Some genes are selected, and some are neutral markers. The inversion is always neutral. Selection can be on haploid survival (fitnesses 1:1-s) or on diploids (1:1-hs:1-s). Fitnesses multiply across loci. The heterozygote advantage should only appear if deleterious genes are recessive ($h<0.5$). The limit for N is that for diploid selection, a table has to be made of all the possible pairs of

haplotypes in a population: this has 2N(2N-1)/2 entries. In practice, populations of up to 60 diploid individuals can be simulated. The only limit on n is the longer time necessary for simulations as n increases. On the basis of this consideration, 200 loci were modelled in this study. There are around 10^4 loci in the *D. melanogaster* genome, therefore there are about 4000 (40% of genome) on the third chromosome and about 664 (16.6% of third chromosome) within *In(3R)P*. So modelling 200 loci is the correct order of magnitude to be relevant to the dynamics of *In(3R)P* but is about 30% of the actual number.

The haploid DNA content of *D. melanogaster* is 170000 kb, of which 39.1% makes up the 3rd chromosome: 66470 kb. In *Drosophila* the recombination rate is roughly $2*10^{-5}$ map units per kb of DNA (Hartl and Clark, 1989, Chapter 7). This means the third chromosome corresponds to $66470*(2*10^{-5}) = 1.3294$ map units (or centiMorgans), and that *In(3R)P* which includes about one sixth of the third chromosome corresponds to 0.2216 map units. As mentioned above the inversion must also include around 664 loci. This gives an adjusted recombination rate between loci, for the area within the inversion of 0.2216/664 = 0.00033. Adjusting for the area within the simulated inversion gives 0.2216/200 = 0.00111 between simulated loci.

About 2% (200/10000) of the Drosophila genome or about 30% of *In(3R)P* are represented by the 200 loci in the simulated inversion. Estimates of μ per gene within *In(3R)P* are between 1 and $4*10^{-6}$ and it is possible to scale these estimates appropriately for the simulated inversion (Voelker *et al*, 1980). Alternatively one can scale from estimates of the total genomic mutation rate. This gives more realistic estimates for natural populations as it includes mutational events other than point mutations, for example transposable element activity. The most recent estimate of the genomic mutation rate for fitness in *D. melanogaster* is from Houle *et al* (1992). Extrapolating from the second chromosome (which represents a similar proportion of the genome to the third) they concluded that the mutation rate per

haploid genome for fitness was at least 0.25 and was more likely to be several times larger. This estimate was in agreement with previous studies on *Drosophila* (e. g. Charlesworth *et al*, 1990). This corresponds to a mutation rate of 0.25/50 = 0.005 for the purposes of this study (as 200 loci are equal to around 2% of the genome). Various rates were simulated around this value corresponding to genomic mutation rates of 0.1 (adjusted to 0.002), 0.3 (0.006), 0.5 (0.01), 0.7 (0.014) and 0.9 (0.018) mutations per generation. In addition simuations were carried out at a rate equivalent to a genomic rate an order of magnitude lower 0.01 (adjusted to 0.0002) in an attempt to identify a lower bound to the effects seen.

The Houle *et al* (1992) work also gave an estimate of (1-h)s of less than 0.02 where hs is the average decline in heterozygous fitness due to deleterious recessive mutations. In regimes (iii) and (iv) (as described below) this gives values of s=0.02 (since (1-0)s=0.02) and s=0.04 (as (1-0.5)s=0.02) respectively. However for the sake of later comparisons between these two regimes both were simulated with s=0.04. In total four regimes were modelled:

(i) neutral control: h=0.5; s=0
(ii) recessive lethal: h=0; s=1
(iii) deleterious recessive: h=0; s=0.04
(iv) additive deleterious: h=0.5; s=0.04

Comparatively slower drift of the inversion can be taken as indirect evidence of balancing selection: selection to some extent contains the effects of drift. One can compare the rate of drift of the inversion frequency, and of other neutral markers, in two ways. First, the program finds the variance of $\delta p = (p_t - p_{t-1})$ which is the definite change in p per generation due to selection. This would be pq/2N (the variance in p attributable to sampling error or drift) with no selection. Secondly, it finds the total variance $\Delta p = (p_t - p_0)$. If all the fluctuations in p due to drift are uncorrelated, Δp will increase as $var(p) = p_0q_0(1-(1-1/2N)^t)$ (Crow & Kimura, 1970, Chapter 7); the

transformation $(1/t)\log_e(1-var(p)/p_0q_0)$ should equal $\log_e(1-1/2N)$. We may expect this to be larger for neutral markers because of hitch-hiking effects, and smaller for the inversion because of chromosomal heterosis.

The program also finds the mean fitness and variance in fitness over time. The mean fitnesses of the three inversion karyotypes are recorded seperately to indicate marginal heterosis. Data were recorded every 10 generations and there was an initial warm-up period of 50 generations to allow mutations to build up on the chromosomes. All simulations were run for 250 generations after this warm-up. Beyond about 2N generations variation starts to be lost as drift removes rarer alleles. A related problem is that with small population size and weak selection per locus, deleterious genes can become fixed by drift, so the mean fitness of the population declines to extinction. However an indication of this phenomenon would be given by the variation in fitness over time.

The default settings for simulation parameters were as follows:
number of replicate populations: 20;
number of diploid individuals in each population: 60;
number of loci per individual: 200;
25% of genes within inversion neutral;
warm-up generations: 50;
additional time: 250 generations;
populations sampled every 10 generations;
recombination rate between genes: 0.00111.

Results

The effect of mutation rate

The default settings were used in combination with six haploid genomic mutation rates (as discussed above) from 0.0002, 0.002, 0.006, 0.01, 0.014 and 0.018. For each mutation rate 20 replicate populations were simulated under each selective regime (see above). It was then possible to plot the average rate of drift (or

-2N*ln[1-var[p]/pq]) under each regime against the time in generations. An example is shown in Plot 2 which depicts the results obtained from simulations ran with a mutation rate of 0.002 under all four regimes. The results of these simulations, at each of the six mutation rates, are in Tables 5.0 and 5.1.

Various results are shown in the tables. The rates of drift of the inversion and a neutral marker which are given are in fact the gradients of the trajectories (as in Plot 2) they follow. In an effort to test for significant differences between the drift of the neutral marker and inversion an F-ratio test was carried out on their variances (the variance of the inversion as numerator and that of the marker as denominator) at generation zero (i.e. after the 50 generation warm-up), where it can be assumed that gene frequencies may still be approximately normal. However none of the simulations in this study were found to exhibit significant differences between the variances at this time. (One could do this test at a later point in the simulations but under the regimes with additive deleterious (iv) and recessive lethal (ii) mutations the inversion has sometimes been fixed or lost as early as generation 30.) The tables also show the decline in the average fitness of the whole population ('ΔWbar') and the average fitnesses of the three inversion karyotypes: the inversion homokaryotype, heterokaryotype and the standard homokaryotype.

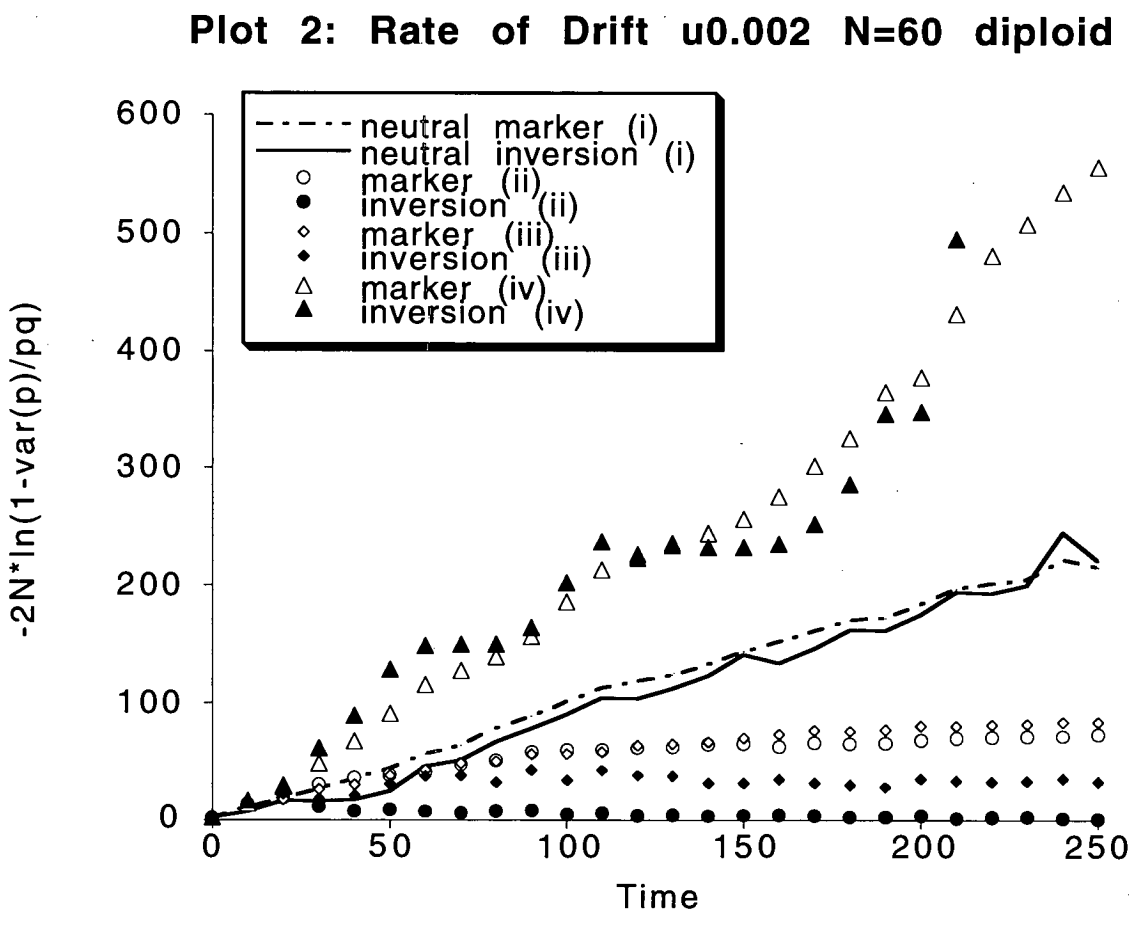
At the first mutation rate (0.0002) the neutral inversion drifts more quickly than the neutral marker, however as mentioned above this difference (in common with any other differences between the inversion and markers' rates of drift) failed to be significant. The inversion drifts more slowly than the marker but both rates are still rising under the regime involving deleterious recessive mutations (iii). There is thus no evidence of long term stability in the frequency of the inversion: eventually it would be lost from the populations or become fixed. Only rather mild heterosis was observed which was apparently incapable of confining the drift of the inversion. This is explicable by the fact

Table 6.0: Populations of 60 diploid individuals (see text for explanation). (Asterisks indicate an absence of heterokaryotypes caused by fixation of one or other arrangement.)

μ	Regime	Rate of drift		Karyotypic Wbar			ΔWbar
		inv	m	s/s	i/s	i/i	
0.0002	(i)	1.427	1.103	1	1	1	0
	(ii)	1.028	1.376	0.972	1	0.970	-0.010
	(iii)	0.462	0.651	0.915	0.992	0.910	-0.070
	(iv)	0.703	1.487	0.910	*	0.931	-0.036
0.002	(i)	0.945	0.905	1	1	1	0
	(ii)	-0.041	0.227	0.015	0.809	0.036	-0.238
	(iii)	0.059	0.301	0.250	0.607	0.309	-0.489
	(iv)	1.728	2.146	0.260	*	0.288	-0.412
0.006	(i)	1.427	1.103	1	1	1	0
	(ii)	-0.011	0.144	0	0.426	0	-0.52
	(iii)	-0.018	0.319	0.031	0.080	0.032	-0.031
	(iv)	6.169	2.765	0.033	*	0.029	-0.309

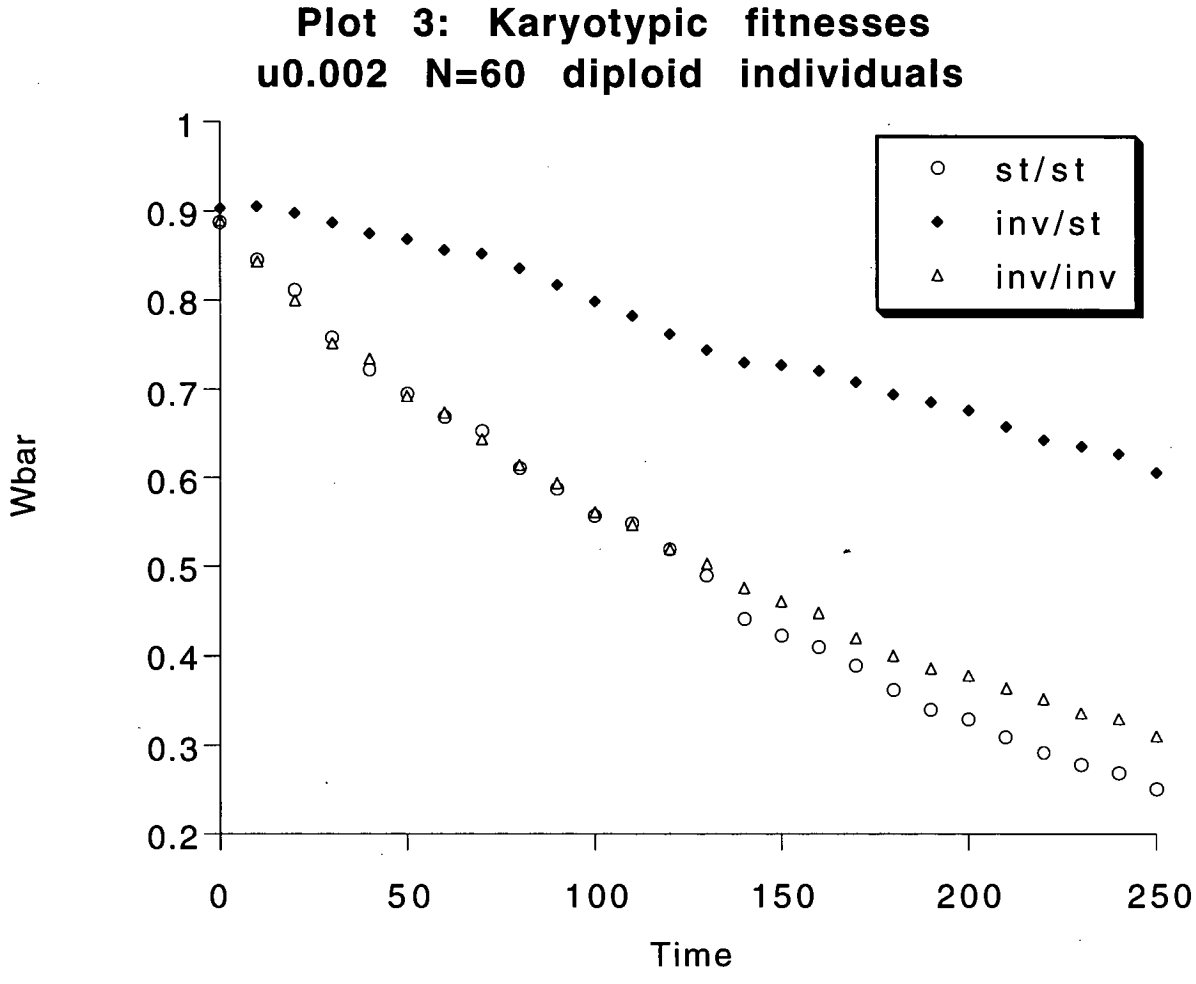
Table 6.1:Populations of 60 diploid individuals.

μ	Regime	Rate of drift		Karyotypic Wbar			DWbar
		inv	m	s/s	i/s	i/i	
0.01	(i)	0.826	0.933	1	1	1	0
	(ii)	0.004	0.125	0.009	0.206	0.010	-0.062
	(iii)	0.095	0.369	0.009	0.017	0.011	-0.413
	(iv)	0.767	1.756	0.008	*	0.008	-0.155
0.014	(i)	1.792	1.177	1	1	1	0
	(ii)	-0.005	0.106	0	0.126	0.003	-0.016
	(iii)	0.276	0.558	0.006	0.007	0.005	-0.260
	(iv)	4.560	2.812	0.004	*	0.004	-0.081
0.018	(i)	0.875	0.989	1	1	1	0
	(ii)	0.001	0.095	0	0.071	0.005	-0.013
	(iii)	0.389	0.606	0.004	0.004	0.004	-0.169
	(iv)	4.578	2.090	0.003	*	0.003	-0.047


Plot 2: Rate of Drift u0.002 N=60 diploid
neutral marker (i)
neutral inversion (i)
marker (ii)
inversion (ii)
marker (iii)
inversion (iii)
marker (iv)
inversion (iv)
-2N*ln(1-var(p)/pq)
600
500
400
300
200
100
0
0
50
100
150
200
250
Time

that the selected deleterious genes mean p rose to only 0.03 by the last generation. Accordingly, the average population fitness falls by only a small amount. A similar situation was seen with lethal mutations (ii) where the inversion and marker drift at a similar rate until the inversion slows, presumably under the influence of mild heterosis. However both the inversion and markers' rates continue to rise. Under regime (iv) with additive deleterious mutations the inversion again drifts more slowly than the marker. This time this is attributable to the fixation of the inversion in 15, and its loss in 5 of the 20 populations. This is the cause of the lack of heterokaryotypes in Table 5. Again the population mean fitness stays about the same. In summary it would seem that this mutation rate was incapable of causing a build up of deleterious mutations fast enough to overcome drift.

Increasing the mutation rate by an order of magnitude (to 0.002) changed the model's behaviour substantially (illustrated in Plot 2). Under (iii) the mean frequency of the selected, deleterious recessive genes rose steadily to 0.3 and the development of associative overdominance was seen. The inversion drifted more slowly than the marker, both stabilising at low drift and strong heterosis developed. At the same time however all karyotypes declined in fitness (see Plot 3): presumably as a result of an increasing number of mutations becoming fixed on the inverted and non-inverted sequences. The population mean fitness also declined from 0.89 to 0.49. Similar, though more exaggerated, behaviour was seen with recessive lethals (ii); both marker and inversion stabilised at low drift. For the inversion this level of drift was almost zero with the karyotypic fitnesses almost reaching balanced lethality. Again, population mean fitness also dropped. The fastest drift was seen additive deleterious mutations (iv) where the marker and inversion drifted at similar rates until the inversion stoped abruptly at generation 220 as 8 populations lost it and 12 fixed it. So it would seem that this level of mutation could cause chromosomal heterosis. Such heterosis did not seem stable though, as it was increasingly eroded by drift i.e. as populations became extinct.


Plot 3: Karyotypic fitnesses
u0.002 N=60 diploid individuals
st/st
inv/st
inv/inv
Wbar
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
50
100
150
200
250
Time

Increasing the mutation rate to 0.006 gave similar results to the previous rate. In this case the mean frequency of deleterious genes rose from 0.2 to about 0.7 over 250 generations with deleterious recessives (iii). As expected under this regime the inversion drifted less than the marker but both rates became stable at low drift. Also as expected, heterosis for the inversion developed but this time had almost decayed completely by generation 250. This effect was again attributable to all karyotypic fitnesses declining, and was presumably caused by the fixation of deleterious mutations. Unsurprisingly, population mean fitness declined substantially. Under lethality (ii) the inversion and marker also stabilised at low drift, with the inversion near zero drift. The karyotypic fitnesses indicated that this was the result of the development of a balanced lethal system. The rapid drift seen under additivity (iv) was again more pronounced for the inversion until it abruptly ceased to drift at generation 90; 12 populations became fixed for it and 8 lost it. For the first time the marker also stopped drifting abruptly at generation 150 having been fixed in 14 populations and lost from the other 6.

At the next mutation rate simulated (0.01) the mean frequency of deleterious recessive genes increased further under (iii), and the effects seen above began to disappear. Although the inversion drifted more slowly than the marker both rates failed to stabilise, and continued to rise gradually. Any mild heterosis that developed was gone by generation 100 as all karyotypes' fitnesses tended towards zero. These effects intensified at higher mutation rates (0.014 and 0.018) and the inversion and marker drifted at progressively higher rates, although the inversion always drifted more slowly than the marker (see Table 5.1). In addition the heterosis became even shorter lived, decaying by generation 30 at a mutation rate of 0.018 due to the decrease in all karyotypes' fitnesses. Under lethality (ii) rates of drift of the marker and inversion stabilise more quickly as the mutation rate rose, and the degree of advantage to heterokaryotypes declined. The population mean fitness also declined, to 0.10 at a mutation

rate of 0.01, 0.06 at 0.014 and 0.03 at a rate of 0.018. With additive deleterious mutations (iv) the trend was for the inversion to drift progressively faster to fixation or loss the higher the mutation rate. In addition, fewer populations became fixed for the inversion: 8 by the time the rate was 0.018. The effect of mutation rate on the rate of drift of the inversion under the regime involving deleterious recessive mutations (iii) is summarised graphically in Plot 4.

In general then it would seem that it is not possible for associative overdominance to develop at a rate of mutation below 0.002, at this population size. Below this level mutations could not build up on either sequence to a level sufficient to cause heterosis and contain the drift of the inversion. Mutation rates of 0.002 up to 0.006 were sufficient to cause heterosis and consequently to maintain the inversion within the populations. However such heterosis proved to be unstable, as these mutation rates were also sufficiently high enough to make the fixation of deleterious genes possible which would eventually lead to population extinction. At rates of mutation above 0.006 such heterosis became even more unstable and short-lived, due to the increased fixation of deleterious genes.

The effect of population size

Three population sizes were employed: 60, 30 and 15 diploid individuals, at each of three mutation rates. The two mutation rates, 0.002 and 0.006, at which evidence of heterosis had been found and 0.0002. This lower rate was investigated to see whether lowering population size could cause the development of heterosis at a mutation rate at which there was none with larger populations.

30 diploid individuals

The results for populations of this size can be seen in Table 5.2. Starting with the lowest mutation rate (0.0002), as expected the

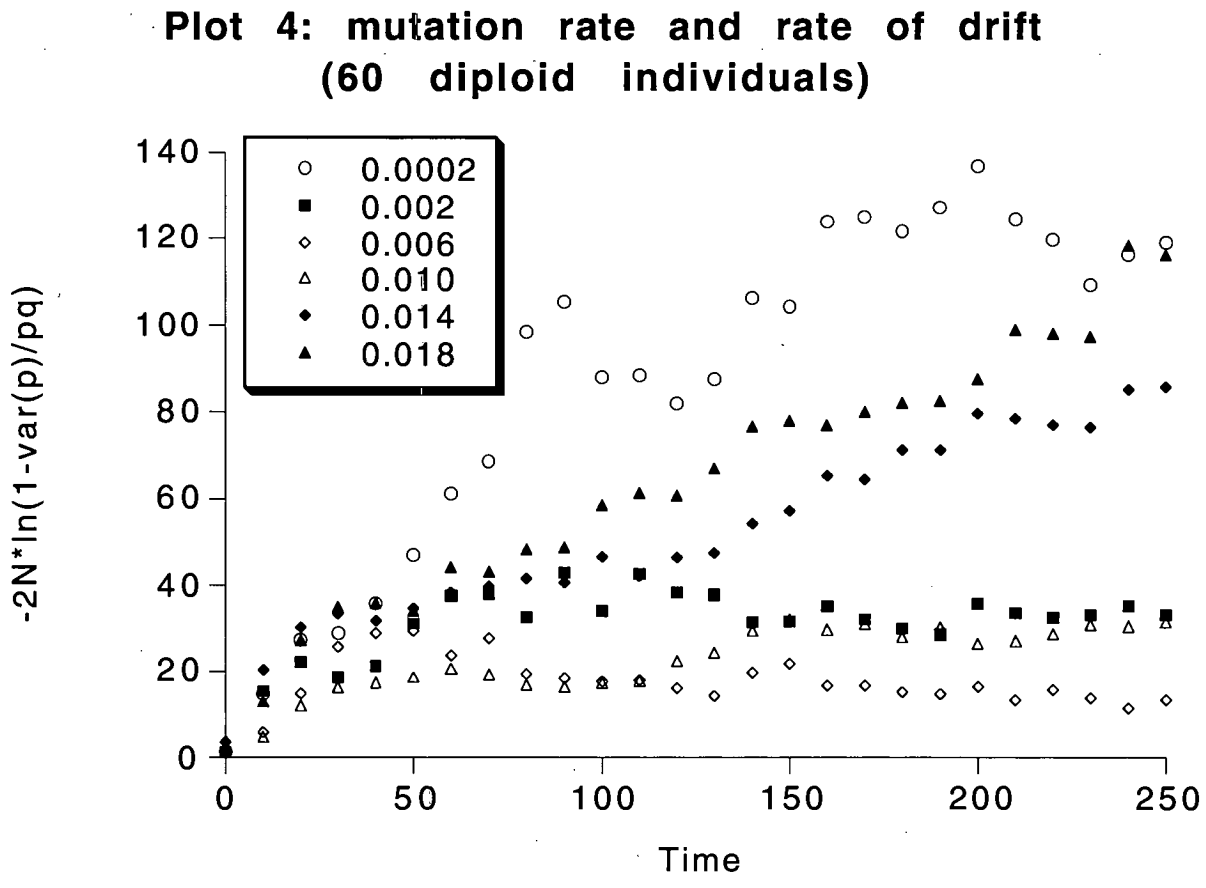

Plot 4: mutation rate and rate of drift
(60 diploid individuals)
0.0002
0.002
0.006
0.010
0.014
0.018
-2N*ln(1-var(p)/pq)
Time
0
20
40
60
80
100
120
140
0
50
100
150
200
250

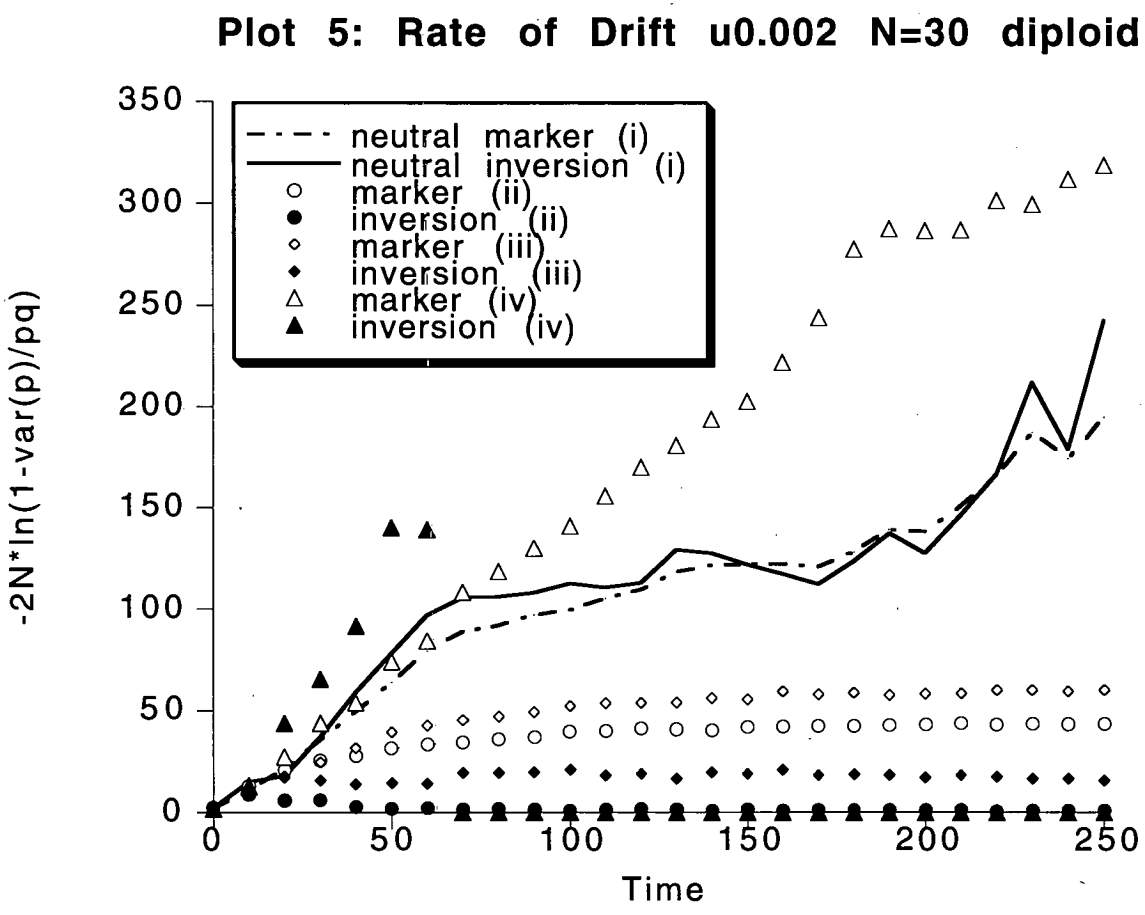
Table 6.2:Populations of 30 diploid individuals.

μ	Regime	Rate of drift		Karyotypic Wbar			DWbar
		inv	m	s/s	i/s	i/i	
0.0002	(i)	0.965	1.365	1	*	1	0
	(ii)	2.292	1.921	0.964	*	0.975	-0.005
	(iii)	0.645	0.596	0.856	0.999	0.879	-0.109
	(iv)	1.389	1.114	0.885	*	0.865	-0.076
0.002	(i)	0.667	0.656	1	1	1	0
	(ii)	-0.017	0.114	0	0.828	0	-0.177
	(iii)	0.025	0.184	0.157	0.540	0.199	-0.537
	(iv)	2.552	1.343	0.206	*	0.206	-0.469
0.006	(i)	0.610	0.648	1	1	1	0
	(ii)	-0.002	0.052	0	0.415	0.021	-0.058
	(iii)	-0.001	0.471	0.028	0.056	0.019	-0.541
	(iv)	2.364	2.586	0.020	*	0.019	-0.280

deleterious genes in the population reached a mean frequency of 0.03 with deleterious recessives (iii), as with the higher population size. However the inversion and marker drifted at similar rates and had stabilised around generation 150. This was accompanied by the fixation of the marker by generation 200 and the development of heterosis associated with the inversion. The behaviour of the marker was mimicked by the marker and inversion under neutrality (i): both became fixed in about half of the populations by generation 230 (and lost from the others). The inversion also went to fixation with lethal recessives (ii) in 8 populations by generation 150 (there was no evidence of heterosis); and under additivity (iv) in 9 populations by generation 170. It was lost from the populations that did not fix it.

At a mutation rate of 0.002 the deleterious genes in the populations with deleterious recessives (iii) reached a mean frequency of 0.3 (the same as with populations of 60). The inversion drifted less than the marker under these conditions but both rates became quite stable and pronounced heterosis developed but with the fitnesses of all karyotypes declining. The population mean fitness declined considerably from 0.85 to 0.32. Stability in the inversion and markers' rates of drift also came about with lethal recessives (ii) but the inversion's drift was almost zero. By generation 250 there was a clear system of balanced lethality. Under the additive regime (iv) the inversion drifted faster than the marker and had been fixed in 11 populations and lost from 9. For comparison with Plot 2 (the same mutation rate with 60 individuals per population) these results are depicted in Plot 5.

The last mutation rate to be simulated at this population size was 0.006. Again, as one would expect the mean frequency of deleterious genes rose to about 0.7, the same level as with populations of 60, with deleterious recessives (iii). Also the inversion drifted less than the marker but both became stable as the marker neared fixation and heterosis for the inversion


Plot 5: Rate of Drift u0.002 N=30 diploid
neutral marker (i)
neutral inversion (i)
marker (ii)
inversion (ii)
marker (iii)
inversion (iii)
marker (iv)
inversion (iv)
-2N*ln(1-var(p)/pq)
Time
0
50
100
150
200
250
300
350

developed. This heterosis decayed by generation 250 as all karyotypic fitnesses approached zero. As with the last mutation rate, under lethality (ii) the marker and inversion stabilised their drift at a constant rate, the inversion's drift approaching zero. This very low drift was attributable to balanced lethality, which developed almost to the point of population extinction by generation 250. Under additivity (iv) the inversion drifted faster than the marker, with the inversion fixing in 11 populations by generation 90.

15 diploid individuals

The same three mutation rates were simulated with this, even smaller population size. As one would expect, for each mutation rate the mean frequency of deleterious genes reached was the same as in previous simulations at the other population sizes. Generally the trends seen in the populations of 30 became more distinct in populations of 15. The neutral marker and inversion began to drift to fixation or loss more quickly, hence the loss of heterokaryotypes under (i) in Table 5.3. As one would expect smaller population size increased the rate of drift.

The results with deleterious recessives (iii) became ever more sensitive to differences in mutation rate. At a mutation rate of 0.0002 rather weak heterosis did develop but this failed to contain the drift of the inversion. The inversion drifted faster than the marker and became fixed or lost in a small number of populations while others maintained it by weak heterosis, at some cost to the population mean fitness. Eventually drift would overcome this heterosis in most if not all populations. At a mutation rate of 0.002 strong heterosis developed and consequently the inversion drifted less than the marker but both rates became very stable, confined by the heterosis. All karyotypic fitnesses declined but the heterokaryotype did so at around half the rate of the other two. In contrast, with the mutation rate set to 0.006 the inversion drifted less than the marker. Both became very stable due to heterosis but the

Table 6.3:Populations of 15 diploid individuals.

μ	Regime	Rate of drift		Karyotypic Wbar			DWbar
		inv	m	s/s	i/s	i/i	
0.0002	(i)	0.909	0.639	1	*	1	0
	(ii)	0.056	0.872	0.980	*	0.958	-0.009
	(iii)	1.455	0.438	0.808	0.922	0.814	-0.170
	(iv)	1.839	0.163	0.827	*	0.809	-0.136
0.002	(i)	1.495	1.256	1	*	1	0
	(ii)	-0.004	0.041	0	0.836	0	-0.105
	(iii)	0.025	0.109	0.132	0.450	0.155	-0.595
	(iv)	0.308	1.829	0.144	*	0.150	-0.482
0.006	(i)	0.942	1.090	1	*	1	0
	(ii)	0.004	0.026	0.042	0.406	0.034	-0.092
	(iii)	-0.006	0.056	0.012	0.030	0.011	-0.455
	(iv)	0.007	0.077	0.014	0.032	0.013	-0.489

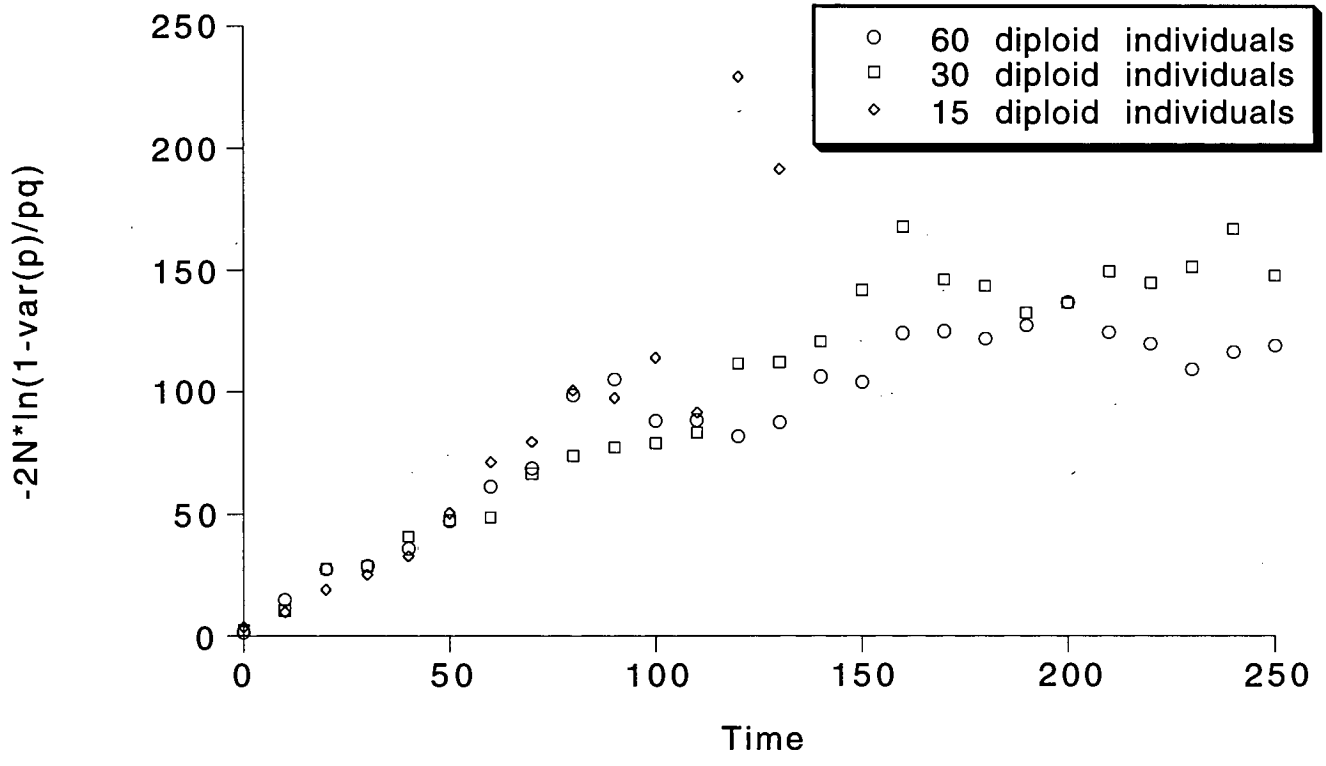
karyotypic fitnesses declined almost to population extinction by generaton 250. The differences in rate of drift under (iii) between different population sizes at each of the three mutation rates are depicted in Plots 6, 7 and 8.

With recessive lethal mutations (regime (ii)) there was a similar pattern. At the lowest mutation rate (0.0002) the marker and inversion both succumbed to drift (and were fixed or lost from every population) by generation 170, despite mild heterosis. With the next mutation rate (0.002) marker and inversion were both maintained at low levels of drift by balanced lethality, which began to erode towards population extinction. The same situation was observed at a mutation rate of 0.006 with one exception. When balanced lethality evolved the heterokaryotype fitness was about half of the fitness achieved with the previous mutation rate. That is the populations were nearer to extinction.

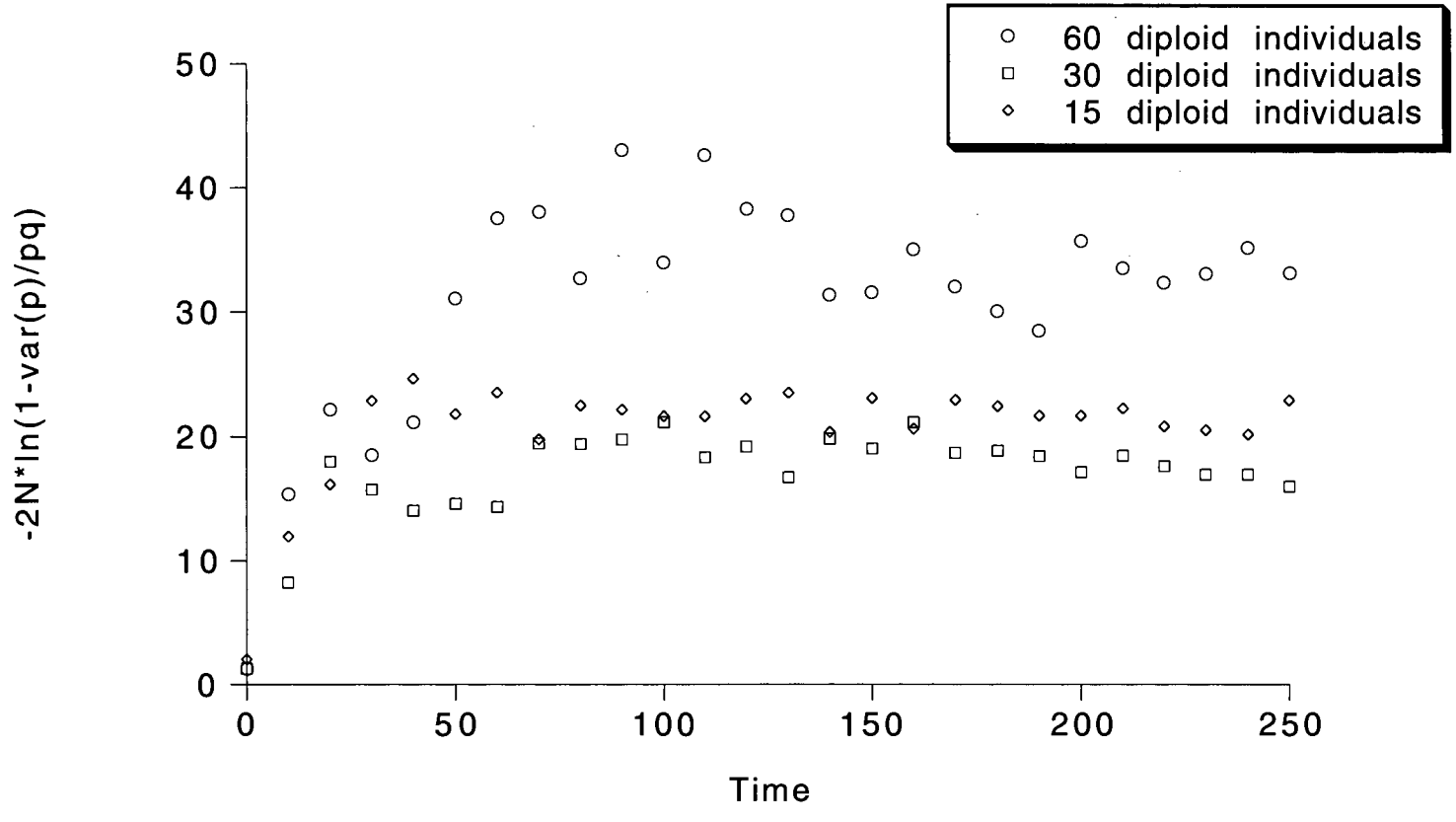
Additivity, under (iv), as has already been observed increased the effectiveness of selection against deleterious genes. At a mutation rate of 0.0002 the inversion drifted faster than the marker, presumably because of associations with deleterious genes (see Charlesworth *et al*, 1993). By generation 80 the inversion had been fixed in 14 populations and lost from the rest. With a mutation rate of 0.002 the inversion became fixed in 7 populations by generation 70. With the mutation rate equal to 0.006 fixation and loss of the inversion had already occurred in the 50 generation warm-up in a number of populations. Interestingly, some mild heterosis appeared to develop but inspection of the population mean fitness (which was only 0.5 at generation zero and rapidly declined) revealed that all populations were close to extinction.

The results on population size and rate of drift suggested two forces at work under regime (iii). Drift helped deleterious mutations to build up on the inverted and non-inverted sequences. At sufficiently high mutation rates this process resulted in associative overdominance and the containment of

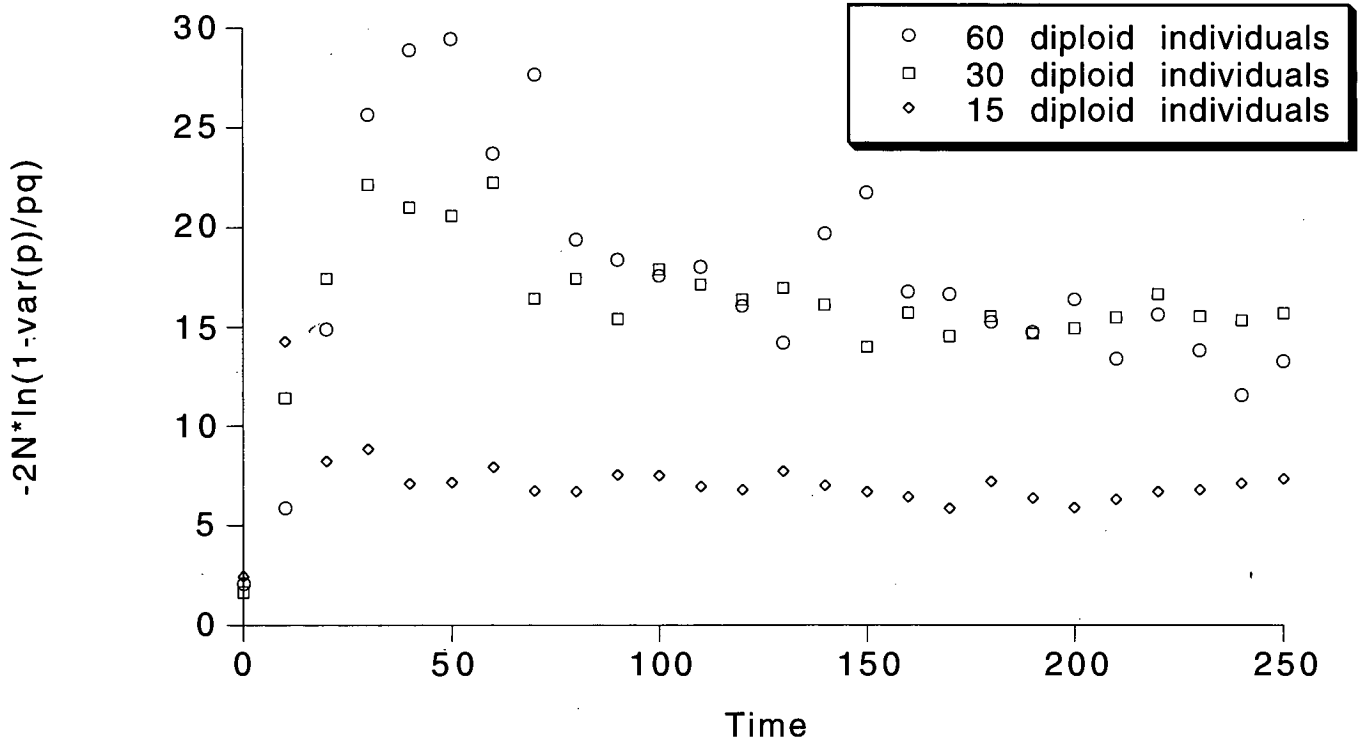
Plot 6: Population size and rate of drift of inversion (mutation rate = 0.0002)



Plot 7: Population size and rate of drift of inversion (mutation rate = 0.002)



Plot 8: Population size and rate of drift of inversion (mutation rate = 0.006)



drift. In contrast, at higher population sizes drift was enhanced by 'hitch-hiking' due to the removal of deleterious mutations by selection ('purging'). Thus, as population size declined the degree of purging decreased and the strength of heterosis increased at all three mutation rates tested.

Discussion

Two major conclusions can be drawn from the results of the simulations. Firstly, for the simulated inversion, the development of appreciable associative overdominance due to deleterious recessive mutations was only possible over a rather narrow range of mutation rates (0.002 to 0.006 mutations per haploid genome per generation). These rates were somewhat lower than estimates from *D. melanogaster* populations (see Houle *et al*, 1992). Under more realistic, higher mutation rates the build up of deleterious genes caused population extinction in less than 100 generations. The second conclusion concerns population size. Decreasing population size reduced the opportunity for the purging of deleterious genes. This allowed the development of associative overdominance at a lower mutation rate (0.0002 mutations per haploid genome per generation).

The experimental results presented in Chapters 3 and 5 suggested a correlation between increased heterosis and decreasing the number of flies used to found the population. There is therefore general agreement between these experimental results and those from the simulations. However, according to the simulated results, it would seem that the experimental populations should have been rather short-lived, as a result of depressing the population mean fitness. Unfortunately no measurements of declines in the population fitness were made, although anecdotally it was observed that the relatively inbred stocks (used in Chapter 3) grew at lower densities and were more easily anaesthetised than the outbred stocks (used in Chapter 5). In any case the simulations suggest that the

polymorphic isofemale stocks used in the experiments should have become extinct merely over the periods of time they have been maintained in the laboratory (conservatively 68 generations for the relatively inbred stocks and 48 for the outbred). Additional phenomena contributing to the observed heterosis must therefore be postulated. The most obvious possibility is that *In(3R)P* is associated with overdominant loci.

The results of the simulations are also in accordance with observations of the 'cage effect' (discussed in the introductions to Chapters 3 and 5), whereby the maintenance of inversion polymorphisms requires a critical number of founding isofemale lines. Populations started with more lines than this number lose the inversion. The simulated data on population size suggest that this may be because of a fine balance between the accumulation of deleterious recesssive mutations and the purging of these genes. This balance was only achieved at certain population sizes as was most clearly seen at a mutation rate of 0.0002 mutations per haploid genome per generation. At a population size of 60 individuals the dominant force was the purging process and either the inverted or non-inverted sequence could rise to fixation. At 30 individuals purging became less effective, made heterosis stronger and the rate of drift of the inversion slowed. At 15 individuals purging was reduced and heterosis intensified further. However this had the consequence that deleterious genes started to become fixed on either sequence and the mean population fitness eventually declined towards extinction. It should be noted that this decline might be slowed by an increase in population size (as more sequences become available to recombine with) caused by initiating a cage population. In certain *D. pseudoobscura* populations the critical number of isofemale lines needed to maintain polymorphism seems to be 24 (of course this corresponds to at least 48 individuals as the females used to establish lines are inseminated): with higher numbers the inversion is lost (Inoue and Watanabe, 1992). Such critical numbers would be expected to vary between populations, according to the mutations carried by them. In view of the

results presented in Chapter 5 the number for these *D. melanogaster* populations is less than or equal to 10 (or at least 20 individuals). It is interesting that in the simulated populations at a mutation rate of 0.0002 the threshold number, 15 is similar.

This study differed from previous modelling (see the introduction to this chapter) by the incorporation of realistic mutation rates rather than some static number of deleterious genes as in Nei *et al* (1967) and Kojima (1967). Also, recombination rate and the effects of deleterious recessive mutations on fitness were restricted to biologically realisable values for the first time. Consequently this study emphasises the inadequacy of chromosomal heterosis to maintain polymorphism by itself at realistic mutation rates for long periods of time. Having said this, maintenance by chromosomal heterosis alone may be more plausible when certain other phenomena are taken into account.

The model could be made more realistic by adding various other factors. For example, with the inclusion of exchange between the inverted and non-inverted sequences (i.e. double crossovers) one would expect that the purging process would be strengthened. (Double crossovers have been estimated to occur at a rate of about 2×10^{-4} (Payne, 1924) in *In(3R)P*.) This might lead to the development of heterosis at higher mutation rates than were seen in this study. Exchange might also be expected to slow the descent towards population extinction once heterosis had arisen. The addition of overdominant loci (as in the studies by Fraser *et al* (1966) and Kojima (1967)) would be expected to increase the likelihood of heterosis associated with the inversion. In a related vein one could alter the fitness function to investigate the 'Kondrashov effect' (negatively synergistic effects of mutations on fitness). Presumably this would lead to the development of heterosis at lower mutation rates.

The relationship between the experimental (Chapters 2, 3 and 5) and simulated data (Chapter 6) was discussed in the last chapter. Generally it would seem that chromosomal heterosis alone is capable of maintaining the *In(3R)P* polymorphism, especially at lower population sizes, but would rapidly lead to population extinction in nature. However this may be avoided if exchange occurs between different gene arrangements or population size increases. At higher population sizes it is necessary to postulate additional forces behind the maintenance of the polymorphism in nature, as purging makes heterosis ineffective. The experimental results suggest that disassortative mating patterns may play an important role in this.

Relative to the results of the Wilton *et al* (1989) paper the results presented here provide rather more positive evidence for the importance of chromosomal heterosis. The results from Inoue and Watanabe (1992) and others working with populations initiated using different numbers of isofemale lines, are in agreement with the results discussed here.

It is hard to find a better animal system for studying associative overdominance than inversions in *Drosophila*. Especially since new theoretical work suggests it is easier than at first thought for deleterious mutations to build up (Charlesworth, 1994). Exploiting experimental designs similar to Sved's balance equilibration (described in the introduction to Chapter 5) designs may be useful in this respect.

For example, one approach to investigating the basis for heterosis associated with an inversion would be to compete inbred and outbred chromosomes, carrying different chromosomal arrangements, in population cages. The inverted inbred arrangement would be competed against the standard outbred, and the inverted outbred against the standard inbred. If chromosomal heterosis was an important force involved in

polymorphism maintenance then one would predict that the outbred arrangement would enjoy an advantage over the inbred in each case (Barton, 1994: personal communication). Such an approach could be extended to comparisons between the competitive ability of the same arrangements maintained in isofemale lines for different lengths of time. A negative correlation between the degree of competitive advantage experienced by an arrangement and the amount of time spent within an isofemale line could be sought. If found, such a correlation would constitute persuasive evidence for the presence of chromosomal heterosis.

Further experimental studies in this area would also benefit from an experimental design which allowed one to determine karyotype in a less laborious and error prone way. The identification of electrophoretic or molecular markers associated with the inversion would be the obvious way to achieve this. Using such approaches would allow karyotypes to identified at any life stage and remove the error prone inference of fathers. Additional studies of differences between karyotypes in development time and observation of mating between karyotypes would also be desirable.

A major problem encountered during these studies was the restricted availability of appropriate fly stocks. This meant that isofemale lines were used in Chapter 2 whose histories were not fully documented. It would have been desirable to have had some accurate account of the level of inbreeding in these lines. In the absence of direct evidence there are only the anecdotal clues mentioned in the discussion section of Chapter 6.

Once the lines used in Chapter 5 became available it would have been better to compare the ten lines used to construct the outbred populations with relatively inbred populations initiated using flies from only one or two of these same lines. Unfortunately these lines became available too late to allow this, more robust, comparison to be carried out.

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Appendix 1: Procedure for the correction of inferred mating data

The karyotypes of fathers were inferred from eight of the progeny of each female sampled. Unfortunately there may have been differences in zygotic to third instar larval viability between karyotypes (heterosis), as suggested by differences in third instar larval to adult viability. This may have led to the misclassification of heterokaryotype (inv/st) male mates as homokaryotypes. This form of bias would have been particularly severe in matings with inv/st females, due to the absence of one or other homokaryotype in the progeny. For example the chance of missing homokaryotype progeny from a mating between two heterokaryotypes would be $0.75^8 \cong 10\%$ in 8 larvae. With the addition of heterosis such that the two homokaryotypes have viabilities of 0.5 relative to the heterokaryotype then this chance would rise to $(0.5+0.75/0.25+1+0.25)^8 \cong 23\%$. If there were viability differences at the near optimum densities of larvae in the vials used to house individual females, then one would expect to see evidence of it in the karyotypic segregation ratios of the progeny.

The analysis commenced by finding the likelihoods of the three possible paternal karyotypes for each group of eight progeny (or family), given a certain set of karyotypic viabilities, assuming the maternal karyotype was known. These three likelihoods were then weighted by the corrected male mating frequencies (i.e. paternal probability by karyotype) and summed to give the overall likelihood of a family. The (natural) log likelihoods of the families that constitute a data set were then summed and the result was the total log likelihood value for the corrected mating data given a set of viabilities. Heterokaryotype viability was maintained at a standard value of 1 throughout so that total log likelihood could be plotted against possible values of homokaryotype viabilities. The most likely viabilities were then found from the graph. The mating numbers associated with these viabilities could then be compared with those expected if pairing occurs at random (i.e. influenced only by the karyotypic

frequencies in males and females).

The analysis was carried out using iterative functions in *Mathematica* version 2.03 (Wolfram, 1990). These functions are described in more detail below (karyotypes referred to as 0 (st/st), 1 (inv/st) and 2 (inv/inv) throughout):

Karyotypic count function

```
numbers[u_List]:=Map[Count[u,#]&,{0,1,2}];
```

When given a family in the correct form this function simply outputs the numbers of each class in the family. Families are represented as lists and counts as lists of these family lists. For example the family {0,0,1,1,1,1,1,1,2,2} is counted as {2,6,2}.

Inference function

```
classify[u_List,bodge]:=
If[FreeQ[u,0], If[FreeQ[u,2],1,2], If[FreeQ[u,2],0,1]];

classify[u_List,bodge, hom0]:=
If[FreeQ[u,2], If[FreeQ[u,1],0, If[FreeQ[u,0],2,1]];

classify[u_List,bodge, hom2]:=
If[FreeQ[u,0], If[FreeQ[u,1],2, If[FreeQ[u,2],0,1]];
```

The three variants of this procedure are designed to infer male mates from progeny arrays given one of the three female karyotypes. The function FreeQ tests whether a particular form (: karyotype) is absent from a list (:family) and classifies the family according to paternity. For example if the female is known to be inv/st (the first function variant) the father is 0 (st/st) if only 0 or 0 and 1 (inv/st) progeny are present; he is 2 (inv/inv) if only 2 or 2 and 1 are present; and 1 if both 0 and 2 are present.

Error correction function

```
correctedNumbers[{v0_,v1_,v2_},{w0_,w1_,w2_},n_]:=
Module[{err0,err2},
err0=(1-w0/(w0+2w1+w2))^n;(*missing all the 0's*)
err2=(1-w2/(w0+2w1+w2))^n;(*missing all the 2's*)
{v0-v1*err2/(1-err2),
v1(1-err0*err2)/((1-err0)(1-err2)),
v2-v1*err0/(1-err0)}];


correctedNumbers[{v0_,v1_,v2_},{w0_,w1_,_},n_,hom0]:=
Module[{err0,err1},
err0=(w1/(w0+w1))^n;(* missing all the 0's *)
err1=(w0/(w0+w1))^n;(* missing all the 1's *)
{v0-err1*v1/(1-err0-err1),
v1/(1-err0-err1),
v2-err0*v1/(1-err0-err1)}];


correctedNumbers[{v0_,v1_,v2_},{_,w1_,w2_},n_,hom2]:=
Module[{err2,err1},
err2=(w1/(w2+w1))^n;(* missing all the 2's *)
err1=(w2/(w2+w1))^n;(* missing all the 1's *)
{v0-err2*v1/(1-err2-err1),
v1/(1-err2-err1),
v2-err1*v1/(1-err2-err1)}];

correctedFreqs[{v0_,v1_,v2_},{w0_,w1_,w2_},n_]:=
Module[{err0,err2,u0,u1,u2,ss},
err0=(1-w0/(w0+2w1+w2))^n;
err2=(1-w2/(w0+2w1+w2))^n;
u0=v0-v1*err2/(1-err2);
u1=v1(1-err0*err2)/((1-err0)(1-err2));
u2=v2-v1*err0/(1-err0);
If[(u0<0)||(u2<0);
ss={If[u0>0,u0,0],u1,If[u2>0,u2,0]};
```

```
ss=ss/(ss[[1]]+ss[[2]]+ss[[3]])];
```

(NB: the *Mathematica* term 'Module' simply defines a procedure with local variables.)
The first three of these routines calculate the mating numbers corrected for bias, given the observed (inferred) numbers of matings {v0, v1, v2}, and the viabilities {w0, w1, w2} where family size is n. The basis of these calculations are the probabilities of missing each progeny karyotype (err0, err1 and err2), for example err0 is the chance of missing st/st progeny in n larvae according to Mendelian segregation and viability differences. Assuming we know the real proportion of matings for each male karyotype (u0, u1, u2) we can say that for a mating between a inv/st male and female:

$$v0 = u0 + u1(err2(1-err0)/(1-err0err2))$$

In other words the observed st/st male matings will be the real number of st/st male matings plus the number of inv/st male matings wrongly classified as st/st males. The probability of this misclassification will be the chance of missing inv/inv progeny (err2) multiplied by the chance of finding st/st progeny. However the latter chance will include a small chance of finding both st/st and inv/inv progeny so the probability of misclassification is divided by that small chance (1-err0*err2). In the same way we can derive expressions for the observed inv/st and inv/inv male matings:

$$v1 = u1(1-err0)(1-err2)/1-err0err2$$
$$v2 = u2 + u1(err0(1-err2)/1-err0err2)$$

Solving these equations for u0, u1 and u2 gives the expressions used in the correction routine for inv/st female matings (the first variant) above. Similar calculations were done for st/st (the second variant) and inv/inv (the third) female matings.
The last of the routines shown here (correctedFreqs) was developed to remedy a problem which arose in the correction of

inv/st female matings, where the largest misclassification error is possible, namely the calculation of corrected mating numbers (with large viability differences) which were less than one (for homokaryotype males) or greater than the total number of matings for inv/st females (for inv/st males). The routine avoids these problems by truncating the downward correction of matings at zero and by calculating male mating frequencies instead of numbers.

Likelihood function

```
lik[{w0_,w1_,w2_},{n0_,n1_,n2_},0]:=
If[n2>0,0,Multinomial[n0,n1,n2]*
w0^n0*w1^n1/(w0+w1)^(n0+n1)];
lik[{w0_,w1_,w2_},{n0_,n1_,n2_},1]:=
Multinomial[n0,n1,n2]*
w0^n0*(2w1)^n1*w2^n2/(w0+2w1+w2)^(n0+n1+n2);
lik[{w0_,w1_,w2_},{n0_,n1_,n2_},2]:=
If[n0>0,0, Multinomial[n0,n1,n2]*
w1^n1*w2^n2/(w1+w2)^(n1+n2)];
lik[w_List,n_List,{u0_,u1_,u2_}]:=
u0*lik[w,n,0]+u1*lik[w,n,1]+u2*lik[w,n,2];

lik[{w0_,w1_,w2_},{n0_,n1_,n2_},0,hom0]:=
If[(n2>0)||(n1>0),0,1];
lik[{w0_,w1_,w2_},{n0_,n1_,n2_},1,hom0]:=
If[n2>0,0,Multinomial[n0,n1]*
w0^n0*w1^n1/(w0+w1)^(n0+n1)];
lik[{w0_,w1_,w2_},{n0_,n1_,n2_},2,hom0]:=
If[(n0>0)||(n2>0),0,1];
lik[w_List,n_List,{u0_,u1_,u2_},hom0]:=
u0*lik[w,n,0,hom0]+u1*lik[w,n,1,hom0]
+u2*lik[w,n,2,hom0];

lik[{w0_,w1_,w2_},{n0_,n1_,n2_},0,hom2]:=
If[(n0>0)||(n2>0),0,1];
lik[{w0_,w1_,w2_},{n0_,n1_,n2_},1,hom2]:=
```

```
If[n0>0,0,Multinomial[n1,n2]*
w1^n1*w2^n2/(w2+w1)^(n2+n1)];
lik[{w0_,w1_,w2_},{n0_,n1_,n2_},2,hom2]:=
If[(n0>0)||(n1>0),0,1];
lik[w_List,n_List,{u0_,u1_,u2_},hom2]:=
u0*lik[w,n,0,hom2]+u1*lik[w,n,1,hom2]+u2*lik[w,n,2,hom2];
```

This procedure firstly evaluates lik[{w0, w1, w2}, {n0, n1, n2}, i] for each paternal karyotype, which is the chance that a family {n0, n1, n2} with viabilities {w0, w1, w2} came from a father of karyotype i. Subsequently, these likelihoods are weighted according to male mating frequencies and summed to give the chance of the family given these mating probabilities {u0, u1, u2} and the previously incorporated viabilities. The result is a likelihood value for each family in the data set. Again there is one variant of this routine for each female karyotype.

Total log likelihood function

```
totalLogL[ntab_,w_,v_]:=totalLogL[ntab,w,v]=
Sum[Log[lik[w,ntab[[i]],v]],{i,Length[ntab]}];
totalLogL[ntab_,w_,v_,hom_]:=
totalLogL[ntab,w,v,hom]=
Sum[Log[lik[w,ntab[[i]],v,hom]],{i,Length[ntab]}];
```

This last procedure finds the total log likelihood of the viabilities {w0, w1, w2}, assuming that the mating proportions are given by correctedFreqs[{v0, v1, v2}, {w0, w1, w2}, familysize] by summing the logs of the previously calculated likelihoods across families for a data set. Or to put it another way the function finds the total log likelihood for a data set of families in terms of the two homokaryotype viabilities (while assuming that the inv/st viability is 1), and constructs a table containing this information. In this case it is only necessary to have two variants of this function: one suffices for both homokaryotype females' two dimensional likelihood tables.

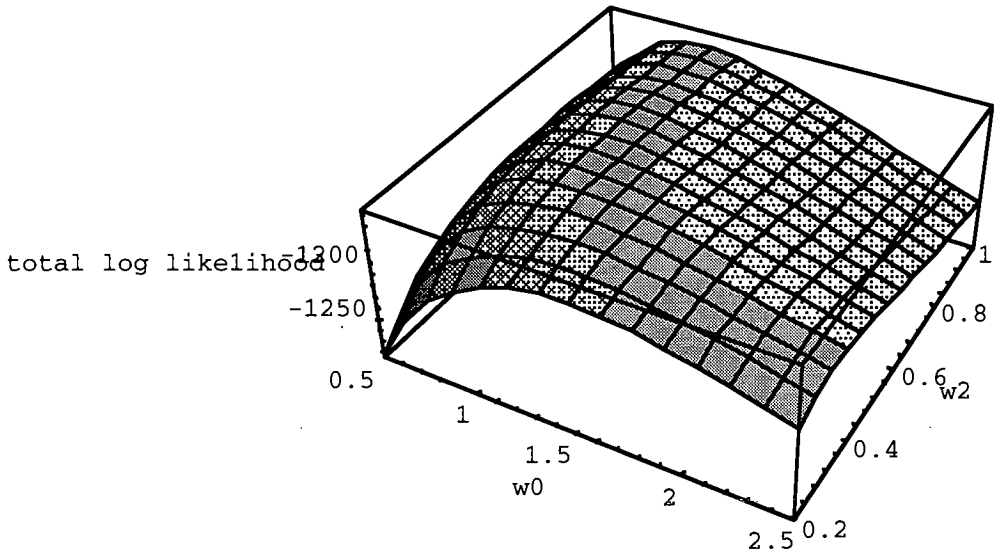
An Example

In practice the error correction, likelihood and total log likelihood functions described above were nested as shown below (note that the likelihood function is already included in the total log likelihood function). The list ntt corresponds to the output from the karyotypic count function. In this example a table of log likelihoods is constructed for the pooled data for inv/st females from Cages 1 to 5 for values of homokaryotype viability between 0.5 and 2.5 for st/st and between 0.2 and 1.5 for inv/inv (values are calculated in 0.2 increments):

```
liktab=Table[{w0,w2,totalLogL[ntt,{w0,1,w2},
correctedFreqs[nobs,{w0,1,w2},8]/148]},
{w0,0.5,2.5,0.2},{w2,0.2,1.5,0.2}]
```

One can then define and plot an approximate function based upon the values in the table using interpolation (which effectively assumes that the function varies smoothly between the known points). In this case it was not necessary to plot the full range of inv/inv viability values to include the peak of the function:

```
linterp=Interpolation[Flatten[liktab,1]];
Plot3D[linterp[w0,w2],{w0,0.5,2.5},{w2,0.2,1}];
Show[%, AxesLabel -> {"w0", "w2", "total log
likelihood"}]
```



It is then possible to find the peak value: -1160.01 and the homokaryotype fitnesses, w0=1.12797 and w2=0.516259 which correspond to it. (All log likelihood values are negative as likelihoods are probabilities and the natural logarithms of numbers less than 1 are negative.) These viabilities can now be used to generate a set of corrected matings involving inv/st females:

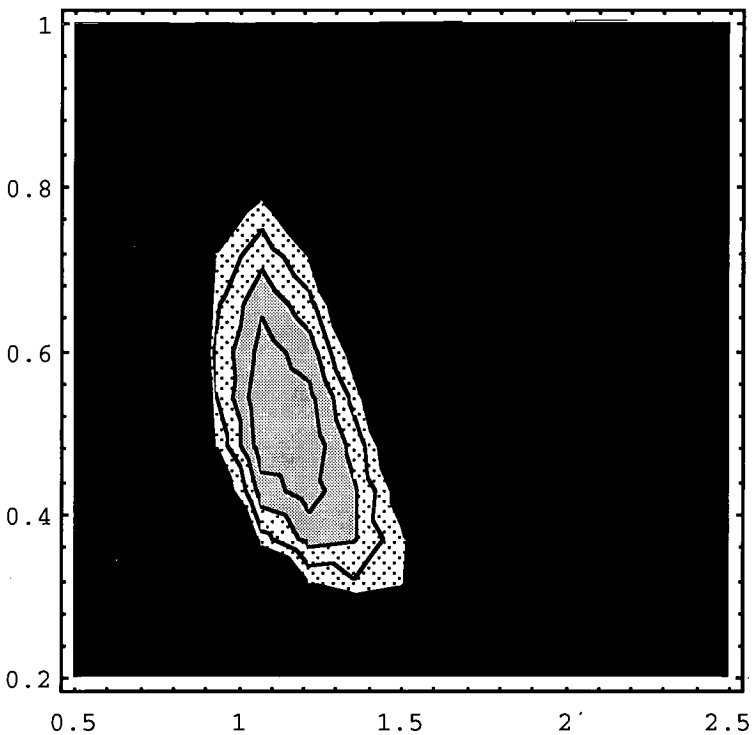
correctedNumbers[{82,56,10},{1.12797,1,0.516259},8]

The correction gives the matings: 58.6, 82.4, 6.9. We can now ask whether there is a significant difference between the highest likelihood value and the value corresponding to no viability differences between karyotypes (i.e. where w0 and w2 both equal 1). There are 2 degrees of freedom as we are estimating two parameters (w0 and w2), so the difference between the the two likelihoods would have to exceed 5.991/2=2.9955 (i.e. half the chi squared distribution critical value) to be significant (p=0.05).

linterp[1,1]+1160.01

In fact the value is 10.214 so there are significant differences in viability between karyotypes.

The confidence limits of the viabilities with the highest likelihood can be estimated by inspection of a contour graph of the area around the function peak, by finding the contour which is furthest from the highest value but is still not significantly different from it. As the contours have been set to be 1 likelihood unit apart the critical value (2.9955) is closely approximated by the third contour (3 units).



So, by inspection (using *Mathematica* observational tools) the confidence limits of w0 (along the horizontal axis) are 0.914 to 1.44, and the confidence limits of w2 (the vertical axis) are 0.322 to 0.748.

This process of deriving functions relating log likelihood to homokaryotype viabilities can be repeated, in two dimensions, for matings involving each of the homokaryotype females.

It is then comparatively simple to combine the functions for the three female karyotypes and again find viability estimates and confidence limits. As the sample sizes for homokaryotype female data are small, it is usually the case that little additional information is given in the combined function (as compared to the inv/st female estimate above) but the confidence limits are narrowed:

w0 = 1.139 (0.955, 1.38); w2 = 0.492 (0.371, 0.672)

Appendix 2: Procedure for the calculation of fitness estimate confidence limits

All viability estimates were calculated in the same way. The number of individuals of a given karyotype at a certain stage of the life cycle (e.g. adult) was divided by the number of individuals present at a previous stage (e.g. larval, for an estimate of larval to adult viability). This constituted a 'naive' viability estimate. Equivalent estimates for male mating success were calculated by dividing the observed matings of a given karyotype (corrected as in Appendix 1) by those expected under random mating.

In order to calculate confidence limits which were comparable to those calculated for zygotic to larval viability in vial populations (see Appendix 1), a similar log likelihood procedure was used. The procedure began with input values for observed numbers of, for example, larvae (when calculating larval to adult viability): {nl0, nl1, nl2}; and observed numbers of adults: {na0 ,na1 ,na2}. The likelihood of the observed frequencies {p0, p1, p2} and various sets of viabilities {w0, 1, w2} given the observed numbers was then found i.e.

L ({p0,p1,p2}, {w0,1,w2}) =
([p0^nl0]*[p1^nl1]*[p2^nl2])*([w0*p0^na0]*[p1^na1]*[w2*p2^na2])/
[w0*p0+p1+w2*p2]^na0+na1+na2

By taking logL we arrive at the only function not used in the procedure described in Appendix 1. This finds the log likelihood of the larval frequencies {p0, p1, p2} and the viabilities {w0, 1, w2} given the observed numbers of larvae {nl0, nl1, nl2} and adults {na0, na1, na2}:

FindLogL function

```
FindLogL[{nl0_,nl1_,nl2_},{na0_,na1_,na2_},
{w0_,1,w2_},{p0_,p1_,p2_}]:=
nl0*Log[p0]+nl1*Log[p1]+nl2*Log[p2]+
na0*Log[w0*p0]+na1*Log[p1]+na2*Log[w2*p2]-
(na0+na1+na2)*Log[w0*p0+p1+w2*p2];
```

In the same manner as in Appendix 1 a three dimensional plot of the function logL for a range of w0 and w2 values was then constructed. The peak log likelihood value from this plot should then correspond to naive estimates i.e. w0: 1: w2 = na0/nl0 : na1/nl1 : na2/nl2. Again, as in Appendix 1, the 95% confidence limits will correspond to the area within 3 log likelihood units of the peak value and can be read from a contour plot of the function.

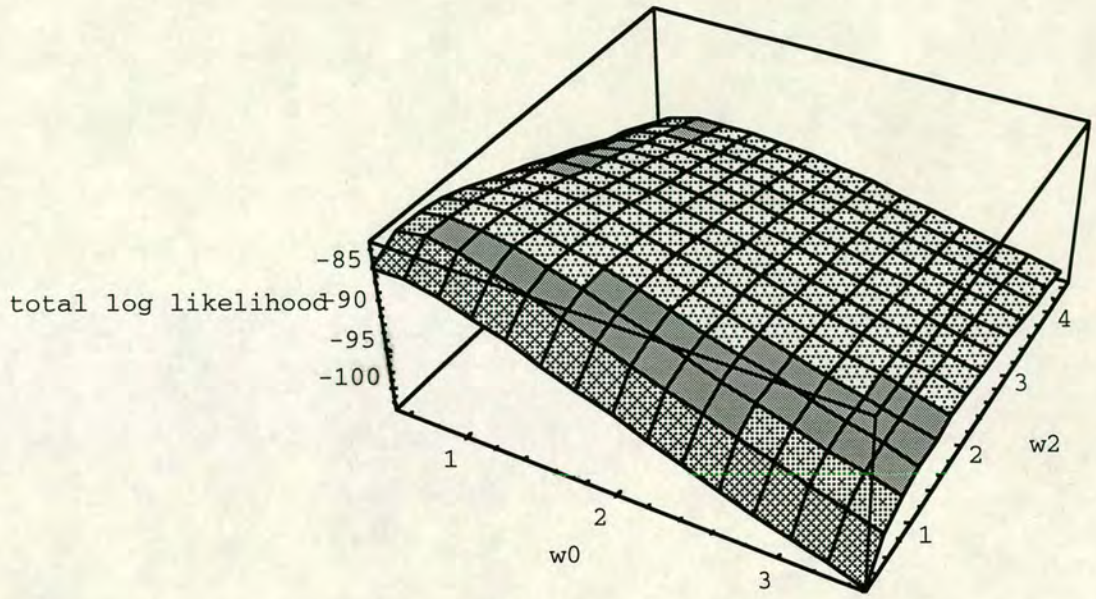
An example

The following example relates to the calculation of confidence limits for zygotic to larval viability in Cage 1 of Chapter 2. After the zygotic (the nl values in the function) and larval (the na values) numbers had been entered a table of log likelihoods is constructed for the data for values of homokaryotype viability between 0.5 and 3.5 for the st/st karyotype and between 0.2 and 4.5 for inv/inv (values are calculated in 0.2 increments):

```
liktab=Table[{w0,w2,FindLogL[nl,na,{w0,1,w2},
nl/Apply[Plus,nl]]},{w0,.5,3.5,0.2},{w2,.2,4.5,0.2}]
```

An approximate function based upon the values in the table was then defined by interpolation (see Appendix 1) and plotted:

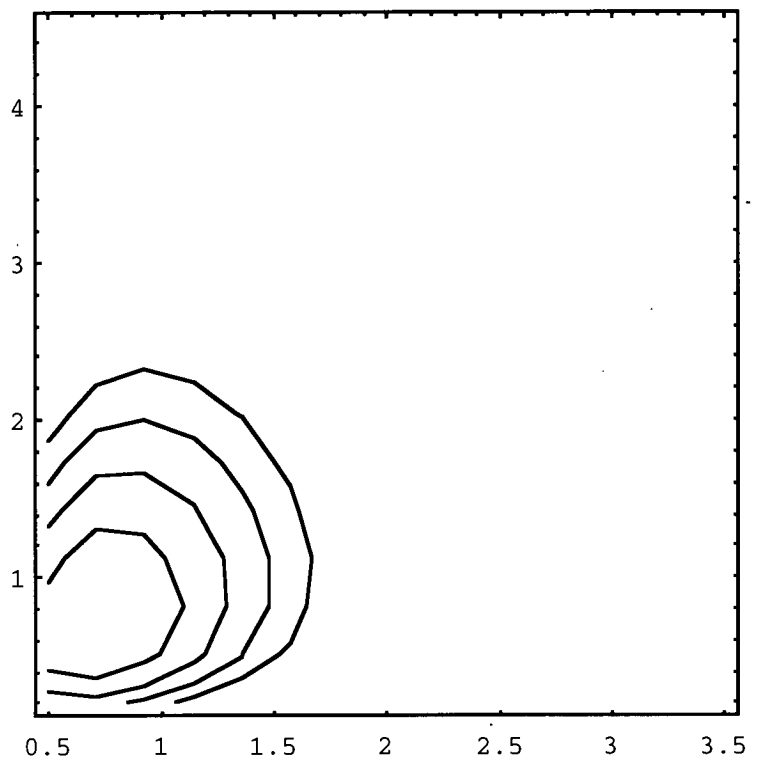
```
linterp=Interpolation[Flatten[liktab,1]];
Plot3D[linterp[w0,w2],{w0,0.5,3.5},{w2,0.2,4.5}];
Show[%, AxesLabel -> {"w0", "w2", "total log
likelihood"}]
```



It was then possible to find the peak value: 82.5952 and the homokaryotype fitnesses, w0: 0.7097 and w2: 0.7032 which correspond to it. Consequently one could ask whether there was a significant difference between the highest likelihood value and the value corresponding to no viability differences between karyotypes (i.e. where w0 and w2 both equal 1). There were 2 degrees of freedom as we were estimating two parameters (w0 and w2), so the difference between the two likelihoods would have to have exceeded 5.991/2=2.9955 (i.e. half the chi squared distribution critical value) to be significant (p=0.05). In fact the value was only 0.7697 so there were no significant differences in viability between karyotypes.

One can compare these results with the naive estimates of w0, w2 and the G-tested significance of the differences between the three karyotypes (see Table 2.12, Chapter 2). The same values for w0 and w2 were found in each case, as well as similar non-significant values for the differences between karyotypes.

As in Appendix 1 the confidence limits of the peak likelihood value were estimated by inspection of the contour plot of the function (additional graphs with different ranges were plotted to obtain the limits not shown below). In other words the contour which was furthest from the highest value but was still not significantly different from it was found. As the contours had been set 1 likelihood unit apart the critical value (2.9955) was closely approximated by the third contour (3 units).



So, by inspection the confidence limits of w0 were 0.321 to 1.48 while the confidence limits of w2 were 0.18 to 2.0.

The drawback of this method was that the confidence limits of certain fitness estimates could not be found, as the computer could not plot them. This applied to estimates of w0 and w2, particularly of male mating success (see Table 2.14), that included zero and one estimate where w0 and w2 varied too widely to be plotted (the male mating success estimates for Cage 2 in Table 2.14). It was also impossible to plot the point where

w0 and w2 both equalled 1 in certain cases, as the estimates for w0 and w2 were so far removed from it. For this reason G values rather than likelihood differences are shown in the fitness estimate tables.

Appendix 3: An experiment to measure the total fitness of wild type third chromosomes in *D. melanogaster*

As discussed in Chapter 2, some mechanisms for the evolution of female mating preferences propose that females choose mates on the basis of their genetic quality, or "good genes" (reviewed by Kirkpatrick & Ryan, 1991). It is said that this would be selectively advantageous to a female because the offspring she produced would also be of relatively high fitness. However it is orthodox in population genetics to assume no additive genetic variance in total fitness, within randomly mating populations under natural selection (Falconer, 1989). Certain factors such as mutation, migration from other populations and changes in selection over time could invalidate this assumption (Partridge, 1983). Unfortunately the existing information on the quantitative genetics of total fitness is insufficient to raise discussion of these effects above speculation. A study investigating the variation in total fitness and its components in large *Drosophila* populations would enhance understanding of sexual selection (Charlesworth, 1987), as well as contributing to our knowledge of adaptation and life history evolution.

This study intended to utilise a modified version of Sved's balance technique for *Drosophila* (Sved and Ayala, 1970), adapted for three chromosomes: the balancers TM1 and TM2, and an extracted lethal wild type (+). As the combinations TM1/TM1, TM2/TM2 and +/+ are lethal, the result was individuals of the genotypes TM1/+, TM2/+ and TM1/TM2 in the population cage. The heterozygous fitness effects of the wild type chromosome could then be measured relative to the TM1/TM2 standard. This would make it possible to calculate the relationship between the heterozygous and homozygous fitness effects of the wild type chromosome (assuming the homozygous fitness to be zero). This process was to be carried out for 24 lethal chromosomes: the lethal fraction of an original random sample of 160 wild type third chromosomes, extracted from

an outbred laboratory Dahomey (Dah) stock. The third chromosome was chosen for study as it represents a large portion (about 40%) of the *Drosophila* genome. It was hoped that this would allow for the fact that fitness heritability is likely to be caused by rare partially recessive alleles present in heterozygotes.

Unfortunately the TM1/TM2 genotype was found to compete badly with the other two, so that the TM1/TM2 frequency tended to zero and the TM2/+ frequency tended to 100% of cage populations. Two alternatives were suggested as means to salvage the study:
1. Injection of TM1/TM2 flies into a population cage containing all three genotypes, and measuring the amount of flies needed to be added for the cage to reach equilibrium.
2. Infection of a cage containing TM1/+ flies with TM2/+ flies, and measuring the time until the TM2/+ genotype dominates.
It would have been desirable to proceed with both of these strategies, as agreement in results between the two would constitute a persuasive arguement in the measurement of correspondence between heterozygous and homozygous fitness effects. However it was soon realised that attempting both strategies would have been prohibitively labour intensive.

Materials and Methods

It was decided to set up six population cages for each of the 24 lethal chromosomes: two replicate cages maintaining pure cultures of TM2/+ flies and another four containing only TM1/+ flies, with the aim of proceeding with option 2 above. The chromosome extraction and breeding programme was as follows.

(160 x) 1 x +/+ (Dah)♂ ⊗ 6/7 x TM1/TM2 (Dah)♀

(160 x) 1 x TM2/+♂ ⊗ 10 x TM1/TM2 (Dah)♀

Isolated 10 x TM1/+♀ and ♂ for lethality testing.

Other flies cultured in bottles for all 160 lines.

200 TM2/+♂ collected from 24 lethal lines for backcross #1 with 200 TM1/TM2 ♀.

Same procedure for backcross # 2.

Same procedure for backcross # 3 (at 16.5°C).

Same procedure for backcross # 4.

Virgin ♀ and ♂ collected for six lines and used to set up cages.

TM2/+ ♂ collected from other 18 lines which undergo backcross # 5.

Virgin ♀ and ♂ collected for next six lines and used to set up cages.

TM2/+ ♂ collected from other 12 lines which undergo backcross # 6.

Virgin ♀ and ♂ collected for next six lines and used to set up cages.

TM2/+ ♂ collected from other 6 lines which undergo backcross # 7.

Virgin ♀ and ♂ collected for last six lines and used to set up cages.

Results and discussion

After preliminary sampling, the majority of the population cages were discovered not to be pure cultures, but had suffered contamination at some point in the breeding programme. Work with the remaining, uncontaminated cages did not continue, as there were too few to continue with the original experiment. Instead the attention of the experimenter moved to the ways in which non-additive variation of the third chromosome might be exploited by sexual selection. Investigations in this area are presented in Chapters 2, 3 and 5.

Appendix 4: Photomicrographs.

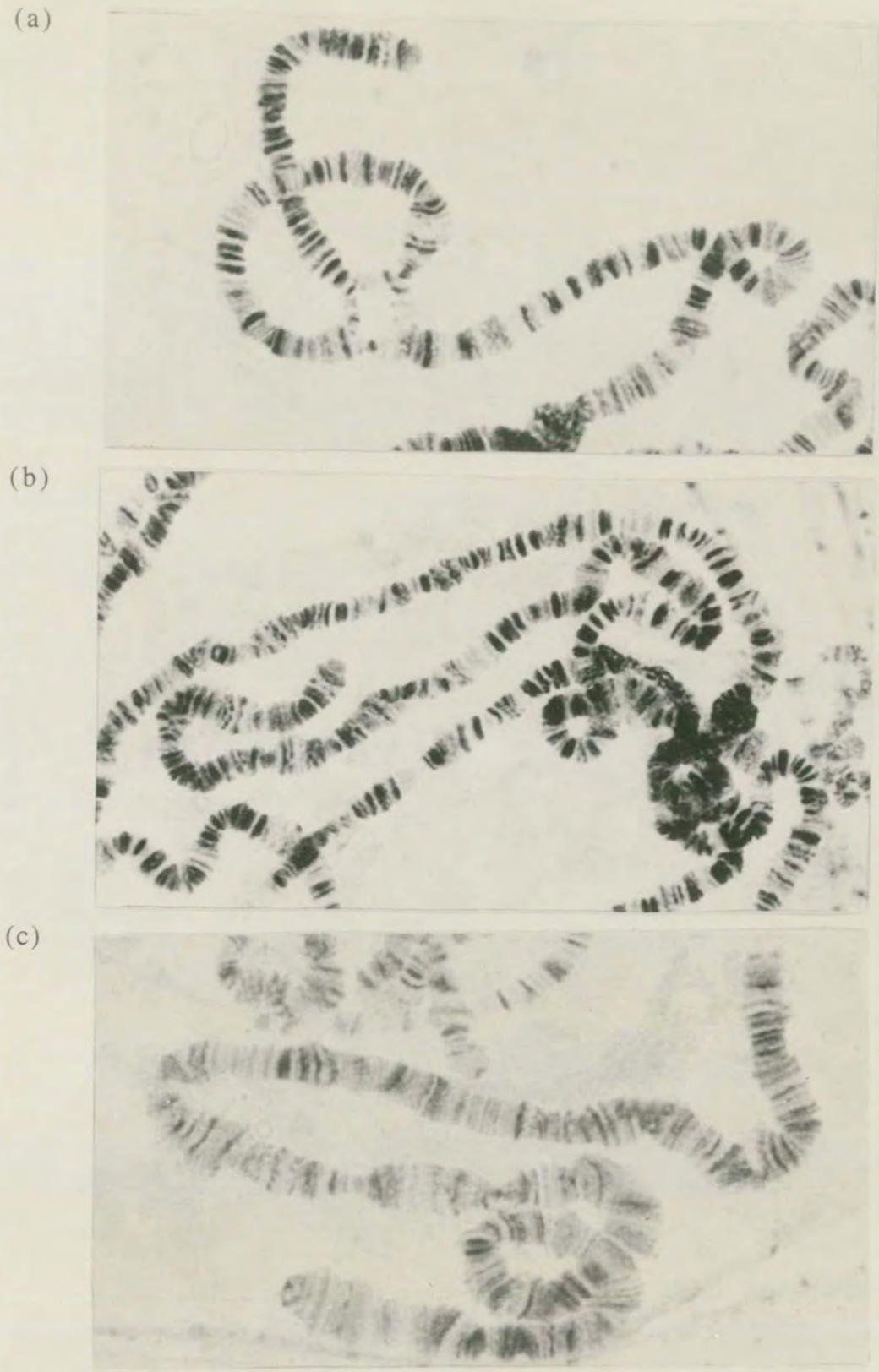


Fig. 1. Photomicrographs of *In(3R)P* in (a) the heterokaryotype, (b) the homokaryotype; and the standard homokaryotype (c).

(a)

(b)

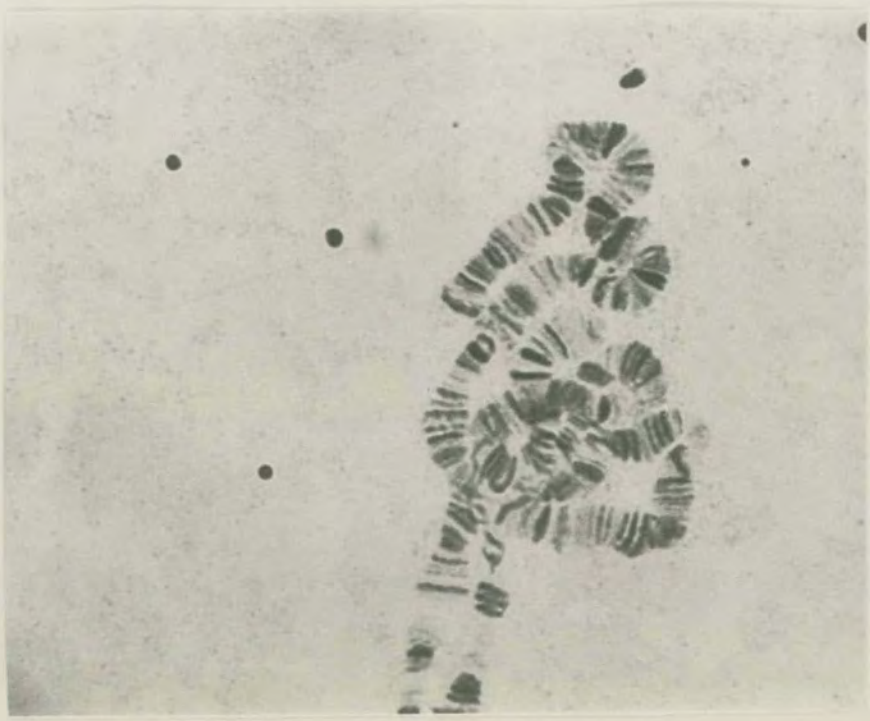
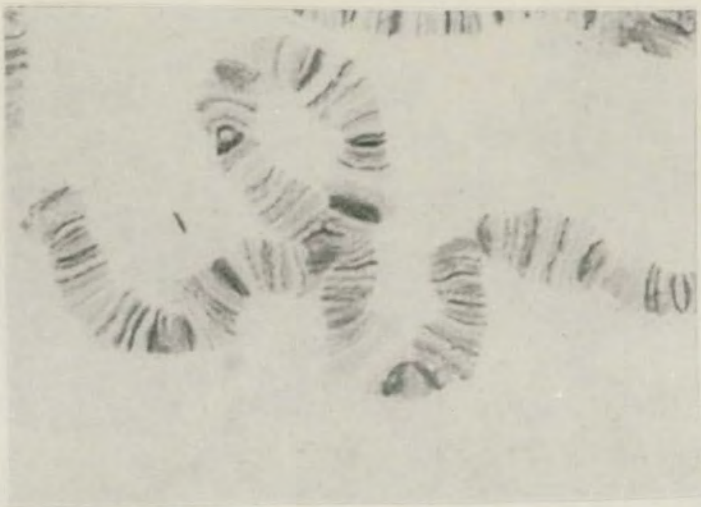


Fig. 2. Photomicrographs of heterozygous second chromosome inversions carried by tester strain (*In(2L)wg^{P} / In(2LR)Gla, Gla*): (a) *In(2L)wg^{P}* ; (b) *In(2LR)Gla.*

(a)

(b)



(c)

Fig. 3. Photomicrographs of (a) *In(2L)t*, (b) *In(2R)NS* and (c) *In(3R)P* heterokaryotypes.